WO 2004/007712

No September 1985

DT89 Rec'd PCT/PTO 1 8 JAN 2005

Title: Modulating developmental pathways in plants.

5

15

25

The invention relates to a method to modulate plant growth or development by modifying genes in plants. The invention among others relates to modifying RKS genes or gene products as found in Arabidopsis thaliana or other plants. The different domains of RKS gene products essentially have the following functions: The first domain of the predicted protein structure at the N-terminal end consists of a signal sequence, involved in targeting the protein towards the plasma membrane. Protein cleavage removes this sequence from the final mature protein product (Jain et al. 1994, J. Biol. Chemistry 269: 16306-16310). The second domain consists of different numbers of leucine zipper motifs, and is likely to be involved in protein protein dimerization. The next domain contains a conserved pair of cystein residues, involved in disulphate bridge formation. The next domain consists of 5 (or in the case of RKS3 only 4) leucine rich repeats (LRRs) shown in a gray colour, likely to be involved in ligand binding (Kobe and Deisenhofer 1994, TIBS 19: 415-420). This domain is again bordered by a domain containing a conserved pair of cystein residues involved in disulphate bridge formation often followed by a serine / proline rich region. The next domain displays all the characteristics of a simgle transmembrane domain. At the predicted cytoplasmic site of protein a domain is situated with unknown function, followed by a domain with serine /threonine kinase activity (Schmidt et al. 1997, Development 124: 2049-2062, WO 01/29240). The kinase domain is followed by a domain with unknown function whereas at the Cterminal end of the protein part of a leucine rich repeat is positioned, probably involved in protein-protein interactions.

Plant homologs of the Arabidopsis RKS genes can be found by comparison of various plant database (see also Table 2) and comprise amongst others:

5 Y14600|SBRLK1|Sorghum bicolor

BF004020|BF004020|EST432518 KV1 Medicago truncatata

AW934655|AW934655|EST353547 tomato

AW617954|AW617954|EST314028 L. pennellii

AA738544|AA738544|SbRLK2 Sorghum bicolor

10 AA738545|AA738545|SbRLK3 Sorghum bicolor

BG595415|BG595415|EST494093 cSTS Solanum tuberosa

AI896277 | AI896277 | EST265720 tomato

BF643238 | BF643238 | NF002H05EC1F1045

AA738546|AA738546|SbRLK4 Sorghum bicolor

15 BE658174|BE658174|GM700005A20D5 Gm-r1070 Glycine max

BF520845|BF520845|EST458318 DSIL Medicago truncata

AC069324|AC069324|Oryza sativa

AW761055|AW761055|s170d06.yl Gm-c1027 Glycine max

BE352622|BE352622|WHE0425 G11 M21ZS Wheat

20 BG647340|BG647340|EST508959 HOGA Medicago truncata

AY028699|AY028699|Brassica napus

AW666082|AW666082|sk31h04.y1 Gm-c1028 Glycine max

AA738547|AA738547|SbRLK5 Sorghum bicolor

BG127658|BG127658|EST473220 tomato

25 L27821|RICPRKI|Oryza sativa

BG238468|BG238468|sab51a09.y1 Gm-c1043 Glycine max

BG441204 | BG441204 | GA Ea0012C15f Gossypium arbo.

AW667985|AW667985|GA _Ea0012C15 Gossypium arbore.

AW233982|AW233982|sf32g05.y1 Gm-c1028 Glycine max

30 <u>AP003235</u>|AP003235|*Oryza sativa*

BF460294|BF460294|074A05 Mature tuber

AY007545|AY007545|Brassica napus

AC087544|AC087544|Oryza sativa

AB041503|AB041503|Populus nigra

35

The invention furthermore relates to modifying ELS genes or gene products or functional equivalents thereof which are for example derived from at least two different genes in the

40 Arabidopsis genome. They show high homology on protein level

with the corresponding transmembrane RKS gene products. However, they lack a transmembrane domain while they do contain a signaling sequence at the N-terminal end. Therefore these proteins are thought to be positioned within vesicles

5 within the plant cell or at the outside of the plasma membrane, within the cell wall of the plant cell. A number of homologs have been detected in other plant species, such as:

AF370543|AF370543|Arabidopsis thaliana

10 AF324989|AF324989|Arabidopsis thaliana

AV520367|AV520367|Arabidopsis thaliana

AV553051|AV553051|Arabidopsis thaliana

BF642233|BF642233|NF050C09IN1F1069

AW559436|AW559436|EST314484 DSIR Medicago truncata

15 BG456991 | BG456991 | NF099F02PL1F1025

AW622146|AW622146|EST312944 tomato

BF260895|BF260895|HVSMEf0023D15f Hordeum vulgare

BE322325 | BE322325 | NF022E12IN1F1088

BG414774|BG414774|HVSMEk0003K21f Hordeum vulgare

20 BE460627|BE460627|EST412046 tomato

BI204894|BI204894|EST522934 cTOS Lycopersicon esculentum

BI205306|BI205306|EST523346 cTOS Lycopersicon esculentum

BI204366|BI204366|EST522406 cTOS Lycopersicon esculentum

AW443205 | AW443205 | EST308135 tomato

25 AW031110|AW031110|EST274417 tomato

BI180080|BI180080|EST521025 cSTE Solanum tuberosa

BF644761 | BF644761 | NF015A11EC1F1084

AV526127|AV526127|Arabidopsis thaliana

AV556193|AV556193|Arabidopsis thaliana

30 BE203316|BE203316|EST403338 KV1 Medicago truncatata.

AW649615|AW649615|EST328069 tomato

BE512465 | BE512465 | 946071E06

BI204917|BI204917|EST522957 cTOS Lycopersicon esculentum

BG590749|BG590749|EST498591

35 BG648725|BG648725|EST510344 HOGA Medicago truncata

BG648619|BG648619|EST510238 HOGA Medicago truncata

BG597757|BG597757|EST496435 cSTS Solanum tuberosa

AW221939|AW221939|EST298750 tomato

BE704836|BE704836|Sc01_

40 BG124409|BG124409|EST470055 tomato

- BF051954|BF051954|EST437120 tomato
- BG320355|BG320355|Zm03_05h01_Zea mays
- AV526624 | AV526624 | Arabidopsis thaliana
- AW933960|AW933960|EST359803 tomato
- 5 AW221278 | AW221278 | EST297747 tomato
 - BE405514 | BE405514 | WHE1212 CO1 F02ZS Wheat
 - BG314461|BG314461|WHE2495 A12 A23ZS Triticum
 - BF258673|BF258673|HVSMEf0016G01f Hordeum vulgare
 - BG262637|BG262637|WHE0938 E03 I06ZS Wheat
- 10 AW030188|AW030188|EST273443 tomato
 - BG653580|BG653580|sad76b11.yl Gm-c1051 Glycine max
 - BG319729|BG319729|Zm03 05h01 A Zm03 Zea mays
 - BF053590|BF053590|EST438820 potato
 - BE454808|BE454808|HVSMEh0095C03f Hordeum vulgare
- 15 BI075801|BI075801|IP1_21_D05.b1_A002
 - BE367593|BE367593|PI1 9 F02.b1 A002 Sorghum bicolor
 - 2e-074 BF260080|BF260080|HVSMEf0021A22f Hordeum vulgare
 - BF627921|BF627921|HVSMEb0006I23f Hordeum vulgare
 - BG598491|BG598491|EST503391 cSTS Solanum tuberosa
- 20 AW038168|AW038168|EST279825 tomato
 - ${\tt BG343258} \, | \, {\tt BG343258} \, | \, {\tt HVSMEg0005D23f} \ \, \textit{Hordeum} \ \, \textit{vulgare}$
 - AW925684|AW925684|HVSMEg0005D23 Hordeum vulgare
 - BG416093|BG416093|HVSMEk0009L18f Hordeum vulgare
 - AW683370 | AW683370 | NF011C09LF1F1069
- 25 BE420108|BE420108|WWS020.C1R000101 ITEC WWS Wheat
 - AW350720|AW350720|GM210009A10F4 Gm-r1021 Glycine max
 - AW616564 | AW616564 | EST322975 L. Hirsutum trichome
 - AW011134|AW011134|ST17B03 Pine
 - BF630746|BF630746|HVSMEb0013N06f Hordeum vulgare
- 30 AW926045|AW926045|HVSMEg0006C10 Hordeum vulgare
 - BE519800|BE519800|HV CEb0021E12f Hordeum vulgare
 - BG343657|BG343657|HVSMEg0006C10f Hordeum vulgare
 - BG933682|BG933682|OV1_16_C09.b1 A002
 - BE433368|BE433368|EST399897 tomato
- 35 <u>AW219797</u>|AW219797|EST302279 tomato
 - BF629324|BF629324|HVSMEb0010N06f Hordeum vulgare
 - BE597128|BE597128|PI1_71_A07.g1 A002
 - AW220075|AW220075|EST302558 tomato
 - AW616639|AW616639|EST323050 L. Hirsutum trichome
- 40 BF645214|BF645214|NF032F11EC1F1094
- AW924540|AW924540|WS1_70_H12.b1 A002

AI775448|AI775448|EST256548 tomato

AW983360|AW983360|HVSMEg0010F15f Hordeum vulgare

BF270171|BF270171|GA Eb0007B13f Gossypium arbor.

BE919631|BE919631|EST423400 potato

5 AW037836|AW037836|EST279465 tomato

BF008781|BF008781|ss79h09.yl Gm-c1064 Glycine max

BF254651|BF254651|HVSMEf0004K05f Hordeum vulgare

BE599797 | BE599797 | PI1_79_H01.gl_A002

BE599026|BE599026|PI1_86_E03.g1_A002

10 R89998|R89998|16353 Lambda-PRL2 Arabidopsis

BG841108|BG841108|MEST15-G02.T3 ISUM4-TN Zea mays

AW307218|AW307218|sf54c07.yl Gm-c1009 Glycine max

AI496325|AI496325|sb05c09.yl Gm-c1004 Glycine max

AJ277703|ZMA277703|Zea mays

15 AL375586 | CNS0616P | Medicago truncatula EST

AW350549|AW350549|GM210009A10A12 Gm-r1021 Glycine max

BE125918|BE125918|DG1_59_F02.b1_A002

BF053901|BF053901|EST439131 potato

BE921389|BE921389|EST425266 potato

20 BE597551|BE597551|PI1 71 A07.b1

BE360092|BE360092|DG1 61 C09.b1_A002

BE660084|BE660084|491 GmaxSC Glycine max

AJ277702|ZMA277702|Zea mays

25 The invention also relates to modifying SBP/SPL gene or products which represent a family of transcription factors with a bipartite nuclear localization signal (The SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE (SBP/SPL) gene family of Arabidopsis thaliana, Columbia ecotype). Upon activation
30 (probably by RKS mediated phosphorylation, the bipartite

(probably by RKS mediated phosphorylation, the bipartite nuclear localization signal becomes linear and available for the nuclear translocation of the protein. Within the plant nucleus, the transcription factor regulates transcription by interaction with specific promoter elements. In Arabidopsis

35 thaliana, this family is represented by at least 16 different members (see following list). In many other plant species, we also identified members of this transcription factor family (See list on page 7).

Functional interaction between RKS and SBP proteins was shown by studies in transgenic tobacco plants in which SBP5 and RKS0 were both overexpressed under the control of an enhanced 35S promoter (data not shown). At the tip of double overexpressing plants, embryo structures appeared whereas in the SBP5 overexpressing plants alone or the RKSO overexpressing plants alone no phenotype was detectable at the root tips of transgenic tobacco plants. These results show that both RKS and SBP proteins are involved together in a signalling cascade, resulting in the reprogramming of developmental fate 10 of a determined meristem. (ref. dissertation: http://www.ub.uni-koeln.de/ediss/archiv/2001/11w1204.pdf; Plant Journal 1997: 12, 2 367-377; Mol. Gen. Genet. 1996: 250, 7-16; Gene 1999, 237, 91-104, Genes and Development 1997: 11, 616-628), Proc. Natl. Acad. Sci. USA 1998: 95, 10306-10311; 15 The Plant Journal 2000: 22, 523-529; Science 1997: 278, 1963-1965; Plant Physiol. Biochem. 2000: 38, 789-796; Cell 1996: 84, 61-71; Annu. Rev. Plant Physiol. Plant Mol. Biol. 1999: 50, 505-537

	name	genetic code
	ATSPL1	At2g47070*
	ATSPL2	At5g43270
	ATSPL3	At2g33810*
25	ATSPL4	At1g53160*
	ATSPL5	At3g15270
	ATSPL6	At1g69170
	ATSPL7	At5g18830
	ATSPL8	At1g02065
30	ATSPL9	At2g42200*
	ATSPL10	At1g27370*
	ATSPL11	At1g27360*
	ATSPL12	At3g60030
	ATSPL13	At5g50570
35	ATSPL14	At1g20980
	ATSPL15	At3g57920
	ATSPL16	At1g76580

^{*} annotation in database not complete and/or correct

In many other plant species, we identified members of this transcription factor family, plant homologs of the Arabidopsis SBP/SPL proteins are for example:

5 AB023037 | AB023037 | Arabidopsis thaliana

BG789832|BG789832|sae56b07.yl Gm-cl051 Glycine max

BG123992|BG123992|EST469638 tomato

BG595750|BG595750|EST494428 cSTS Solanum tuberosum

AF370612|AF370612|Arabidopsis thaliana

10 BF728335|BF728335|1000060H02.x1 1000 - Zea mays

X92079|AMSBP2|A.majus

AW331087|AW331087|707047A12.x1 707 - Mixed adult... 128 zea mays

AJ011643|ATH011643|Arabidopsis thaliana

L34039|RICRMSOA|Oryza sativa

15 AJ011638|ATH011638|Arabidopsis thaliana

AJ011639|ATH011639|Arabidopsis thaliana

AJ132096|ATH132096|Arabidopsis thaliana

BF482644|BF482644|WHE2301-2304 A21 A21ZS Wheat

BF202242|BF202242|WHE0984 D01 G02ZS Wheat

20 BE057470|BE057470|sm58e10.yl Gm-c1028 Glycine max

AJ011628|ATH011628|Arabidopsis thaliana

AJ011629|ATH011629|Arabidopsis thaliana

AJ011617 | ZMA011617 | Zea mays

AJ011637 | ATH011637 | Arabidopsis thaliana

25 AJ011622|AMA011622|Antirrhinum majus

AJ011621|AMA011621|Antirrhinum majus

AJ011635|ATH011635|Arabidopsis thaliana

AJ011623|AMA011623|Antirrhinum majus

BF650908 | BF650908 | NF098D09EC1F1076

30 AJ242959|ATH242959|Arabidopsis thaliana

Y09427|ATSPL3|A.thaliana mRNA

AJ011633|ATH011633|Arabidopsis thaliana

AW691786|AW691786|NF044B06ST1F1000

BE058432|BE058432|sn16a06.yl Gm-c1016 Glycine max

35 AW728623|AW728623|GA Ea0017G06 Gossypium arbore.

BG442540|BG442540|GA Ea0017G06f Gossypium arbo.

AJ011626|ATH011626|Arabidopsis thaliana

AJ011625|ATH011625|Arabidopsis thaliana

AI993858|AI993858|701515182 A. thaliana

40 <u>BG593787</u>|BG593787|EST492465 cSTS Solanum tuberosum

BF634536|BF634536|NF060C08DT1F1065 Drought Medicago

BE806499|BE806499|ss59f10.yl Gm-c1062 Glycine max

AW933950|AW933950|EST359793 tomato

AC008262|AC008262| Arabidopsis

B28493|B28493|T10A24TF TAMU Arabidopsis thaliana

5 AJ011644|ATH011644|Arabidopsis thaliana

AC018364|AC018364|Arabidopsis thaliana

AL092429|CNS00VLB|Arabidopsis thaliana

BE435668|BE435668|EST406746 tomato

BG097153|BG097153|EST461672 potato

10 BE440574|BE440574|sp47b09.yl Gm-c1043 Glycine max

AI443033|AI443033|sa31a08.y1 Gm-c1004 Glycine max

U89496|ZMU89496|Zea mays liguleless1

AW433271|AW433271|sh54q07.y1 Gm-c1015 Glycine max

AW932595|AW932595|EST358438 tomato

15 AW096676|AW096676|EST289856 tomato

AJ011616|ZMA011616|Zea mays

AW036750|AW036750|EST252139 tomato

BF626329|BF626329|HVSMEa0018F24f Hordeum vulgare

AJ011614 | ZMA011614 | Zea mays

20 AJ011642|ATH011642|Arabidopsis thaliana

BE022435|BE022435|sm85h04.yl Gm-c1015 Glycine max

X92369 | AMSPB1 | A.majus

AC015450 | AC015450 | Arabidopsis thaliana

AC079692|AC079692|Arabidopsis thaliana

25 AJ011632|ATH011632|Arabidopsis thaliana

AJ011631|ATH011631|Arabidopsis thaliana

BE455349|BE455349|HVSMEh0097E20f Hordeum vulgare

AJ242960|ATH242960|Arabidopsis thaliana

AJ011610|ATH011610|Arabidopsis thaliana

30 AJ132097|ATH132097|Arabidopsis thaliana

AL138658|ATT209|Arabidopsis thaliana

AJ011615 | ZMA011615 | Zea mays

BE499739|BE499739|WHE0975 Wheat

AW398794|AW398794|EST309294 L. pennellii

35 AJ011618 | ZMA011618 | Zea mays

AW747167|AW747167|WS1 66 F11.b1

AJ011577|ATH011577|Arabidopsis thaliana

AI992727|AI992727|701493410 A. thaliana

BE060783|BE060783|HVSMEq0013F15f Hordeum vulgare

40 BE804992|BE804992|ss34h10.y1 Gm-c1061 Glycine max

BE325341|BE325341|NF120H09ST1F1009

AC007369|AC007369|Arabidopsis thaliana AJ011619|ZMA011619|Zea mays BI099345|BI099345|IP1_37_H10.b1_A002 BI071295|BI071295|C054P79U Populus

- 5 AZ920400|AZ920400|1006019G01.y2 1006 AZ919034|AZ919034|1006013G02.x3 1006 BE805023|BE805023|ss35d09.y1 Gm-c1061 Glycine max
 BG582086|BG582086|EST483824 GVN Medicago truncata
 AJ011609|ATH011609|Arabidopsis thaliana
- 10 BE023083|BE023083|sm90e08.yl Gm-c1015 Glycine max

Furthermore, the invention relates to modifying NDR-NHL-genes or gene products. All proteins belonging to this family contain one (and sometimes even more than one) transmembrane 15 domain. Arabidopsis contains a large number of NDR-NHL genes, such as: aad21459, aaf18257, aac36175, k10d20 (position 40852-41619), aad21460, cab78082, aad21461, aad42003, aaf02134, aaf187656, aaf02133, cab43430, cab88990, cab80950, aad25632, aaf23842, al163812, 20 f20d21-35, t13m11-12, f1e22-7, t23g18, f5d14-4266, t32f12-16, f11f19-11, f11f19-12, f11f19-13, t20p8-13, f12k2, f23h14, k10d20-44043, k10d20-12, t19f11-6, t19f11-5, t10d17-10, f22o6-150, f3d13-5, m3e9-80, t25p22-30, mhf15-4, mhf15-5, mrn17-4, mlf18-9, mgn6-11994, mjj3-9667, f14f18-60, At1g17620 F11A6, At5g11890 , At2g27080 , At5g36970 , mlf18 , At1g65690 F1E22 , At4g01110 F2N1 , At2g35980 f11f19 , At4q01410 F3D13 , At1q54540 F20D21 , At2q46300 t3f17 , At5q21130 , At3g11650 T19F11 , At5g06320 MHF15 , At5g06330 MHF15 , At2g01080 f15b18 , At2g35460 t32f12 , At2g27260 f12k2 , At2g35970 f11f19 , At5q53730 MGN6 , At5q22870 MRN17 , At4q09590 , At3q54200 , At1q08160 30 T6D22 , At5g22200 , At3g52470 , At2g35960 f11f19 , At3g52460 , At5g56050 MDA7, At3g20590 K10D20 , At1g61760 T13M11 , At3g20600 K10D20 , At1g13050 F3F19 , At3g11660 T19F11 , At3g44220 , At1g64450 F1N19 , At3g26350 F20C19 C , At4g05220 , At5g45320 K9E15 , At4q23930 , At4q13270 , At4q39740 , At1q45688 F2G19 W , At5q42860

MBD2 , At1g32270 F27G20 , At4g30660 , At2g45430 f4123 , At4g30650 ,

and

At1g69500 F10D13

35

40 ndr1, At2g27080; T20P8.13, At5g21130, At1g65690, At5g36970,

At1g54540, At5g06320, At5g11890, At1g17620, At3g11650, At2g22180, At5g22870, At2g35980, At2g46300, At4g05220, At2g35460, At2g27260, At4g01410, At5g22200, At1g61760, At3g52470, At5g53730, At4g01110, At2g35960, At3g52460, At4g09590, At2g35970, At3g26350, At3g11660, At3g44220, At1g08160, At2g01080, At5g06330, At5g56050, At3g20600, NDR1, At3g54200, At3g20590, At4g39740, At1g32270 syntaxin, putative, At1g13050, At5g45320, At3g20610, At4g26490, At5g42860, At1g45688, At4g26820

NDR-NHL genes belong to a large family of which one of the first identified is the defence-associated gene HIN1 (Harpin-induced gene). HIN1 is transcriptionally induced by harpins and bacteria, that elicit hypersensitive responses in tobacco. It is thus believed that the genes of the invention also play arole in the hypersensitive reaction. Especially (see also chapter 8) since the genes of the invention bear relation to brassinoid-like responses and since brassinoid pathway compounds have been found to interact in this same defence system in plants. Other plant species also contain members of this large gene family, such as:

Plant homologs of the Arabidopsis NDR/NHL genes:

25 BG582276|BG582276|EST484016 GVN Medicago truncata AV553539|AV553539|Arabidopsis thaliana AC069325|AC069325|Arabidopsis thaliana AV526693|AV526693|Arabidopsis thaliana BG583456|BG583456|EST485208 GVN Medicago truncata 30 AW267833|AW267833|EST305961 DSIR Medicago truncata BE997791|BE997791|EST429514 GVSN Medicago truncata BG580928|BG580928|EST482657 GVN Medicago truncata BF520916|BF520916|EST458389 DSIL Medicago truncata AV544651|AV544651|Arabidopsis thaliana 35 AV543762|AV543762|Arabidopsis thaliana AW559665|AW559665|EST314777 DSIR Medicago truncata BG581012|BG581012|EST482741 GVN Medicago truncata AV552164|AV552164|Arabidopsis thaliana BE999881|BE999881|EST431604 GVSN Medicago truncata

AW031098|AW031098|EST274405 tomato

- AI998763|AI998763|701546833 A. thaliana
- AW219286|AW219286|EST301768 tomato
- BE124562|BE124562|EST393597 GVN Medicago truncata
- AV540371|AV540371|Arabidopsis thaliana
- 5 AV539549|AV539549|Arabidopsis thaliana
 - BG647432|BG647432|EST509051 HOGA Medicago truncata
 - BE434210|BE434210|EST405288 tomato
 - BG725849|BG725849|sae42g02.yl Gm-c1051 Glycine max
 - AP003247|AP003247|Oryza sativa
- 10 BE348073|BE348073|spl1all.yl Gm-cl042 Glycine max
 - AW508383|AW508383|si40c06.yl Gm-r1030 Glycine max
 - AI856504|AI856504|sb40b07.yl Gm-c1014 Glycine max
 - BE556317|BE556317|sq01b07.yl Gm-c1045 Glycine max
 - AA713120|AA713120|32681 Arabidopsis
- 15 AV541531 | AV541531 | Arabidopsis thaliana
 - AI894456|AI894456|EST263911 tomato
 - AW704493|AW704493|sk53g11.y1 Gm-c1019 Glycine max
 - AW219298|AW219298|EST301780 tomato
 - BF425685|BF425685|ss03c11.yl Gm-c1047 Glycine max
- 20 AV422557|AV422557|Lotus japonicus
 - BE190816|BE190816|sn79a08.yl Gm-c1038 Glycine max
 - BG580331|BG580331|EST482056 GVN Medicago truncata
 - AV423251|AV423251|Lotus japonicus
 - AI896088|AI896088|EST265531 tomato
- 25 AV413427 | AV413427 | Lotus japonicus
 - AV426656|AV426656|Lotus japonicus
 - AV416256|AV416256|Lotus japonicus
 - AL385732|CNS0690I|Medicago truncatula
 - AB016877|AB016877|Arabidopsis thaliana
- 30 AV419449 | AV419449 | Lotus japonicus
 - AI486269|AI486269|EST244590 tomato
 - AV411690|AV411690|Lotus japonicus
 - AV419925|AV419925|Lotus japonicus
 - AV418222|AV418222|Lotus japonicus
- 35 AV409427|AV409427|Lotus japonicus
 - AC005287|AC005287|Arabidopsis thaliana
 - AV426716|AV426716|Lotus japonicus
 - AV411791|AV411791|Lotus japonicus
 - BG351730|BG351730|131E12 Mature tuber
- 40 <u>BG046452</u>|BG046452|saa54b12.y1 Gm-c1060 Glycine max AI781777|AI781777|EST262656 tomato

12 BE451428|BE451428|EST402316 tomato

AI772944|AI772944|EST254044 tomato

AI895510|AI895510|EST264953 tomato

AW030762|AW030762|EST274017 tomato

5 AW218859|AW218859|EST301341 tomato

BE203936|BE203936|EST396612 KV0 Medicago truncata

AV410289|AV410289|Lotus japonicus

AW032019|AW032019|EST275473 tomato

AW030868|AW030868|EST274158 tomato

10 AV421824 | AV421824 | Lotus japonicus

BG646408|BG646408|EST508027 HOGA Medicago truncata

AF325013|AF325013|Arabidopsis thaliana

AC007234|AC007234|Arabidopsis thaliana

AW217237|AW217237|EST295951 tomato

15 AC034257|AC034257|Arabidopsis thaliana

AW625608|AW625608|EST319515 tomato

AW031064|AW031064|EST274371 tomato

AF370332|AF370332|Arabidopsis thaliana

AB006700|AB006700|Arabidopsis thaliana

20 AW035467|AW035467|EST281205 tomato

AL163812|ATF14F18|Arabidopsis thaliana

AI896652|AI896652|EST266095 tomato

AI730803|AI730803|BNLGHi7970 Cotton

AW034775|AW034775|EST278811 tomato

25

30

The invention provides the insight that RKS proteins or functional equivalents thereof play part in a signaling complex (herein also called the RKS signaling complex) comprising molecules of RKS proteins, ELS (Extracellular Like SERK) proteins, NDR/NHL proteins and SBP/SPL (Squamosa Binding Protein) proteins, and the corresponding protein ligands (see for example table 3) whereby each of these proteins interplay or act in such a way that modifying genes, or modifying 35 expression of genes, encoding ELS, RKS, NDR/NHL or SBP/SPL, proteins or said ligands may lead to functionally equivalent results (Figure 5. Two-hybrid interaction experiments have for example shown in vitro interaction between RKS 0 and NDRO/NHL28 and members of the SBP/SPL family. Here we show that in vivo the individual components of this signaling 40

complex are regulating identical processes, as based on functional genomics on transgenic plants, overexpressing or co-suppressing single components or combinations of components in this transmembrane signalling complex. ELS gene products are derived from at least two different genes in the Arabidopsis genome. They show high homology on protein level with the corresponding transmembrane RKS gene products.

10

15

20

25

30

35

However, they lack a transmembrane domain while they do contain a signalling sequence at the N-terminal end. Therefore these proteins are thought to be positioned within vesicles within the plant cell or at the outside of the plasma membrane, within the cell wall of the plant cell. A number of homologues have been detected in other plant species (see list on page 3). ELS proteins are involved in the heterodimerizing complex with the RKS transmembrane receptor at the outer membrane site. ELS molecules are either in competition or collaboration with RKS molecules involved in the high affinity binding of the ligand. The signal transmitted from the ligand onto the RKS proteins is then transporter over the membrane towards the N-terminal site of RKS protein, located on the other site of the membrane. The activation stage of the RKS molecule is changed, as a result of transphosphorylation by dimerizing receptor kinase dimerizing partners. Subsequently the signal is transmitted to other proteins, one family of such proteins is defined as the SBP/SPL family of transcription factors, the other family of proteins is represented by the NDR/NHL members.

The different obvious phenotypes created by modifying the RKS gene products could be effected by one process regulating all different effects in transgenic plants.

All the phenotypes observed can be effected by the process of brassinosteroid perception. In chapter 1, RKS genes are clearly involved in plant size and organ size. Loss of RKS expression results in a dwarf phenotype, similar as observed with brassinosteroid synthesis mutants. It was already known in literature that the phenotypes observed from modifying the

RKS genes are also observed when modifying the brassinosteroid pathway genes and/or their regulation, thereby altering the amount and nature of the brassinosteroids in plants.

Literature which describes the phenotypic effects of modifying teh brassionosteroid pathway can, amogst others, be found in: Plant Journal 26: 573-582 2001; Plant Journal 1996 9(5) 701-713, genetic evidence for an essential role of brassinosteroids in plant development; J. Cell Biochem Suppl. 21a 479 (1995); Mandava 1988 Plant growth-promoting brassinosteroids, Ann. Rev. Plant. Physiol. Plant Mol. Biol. 39 23-52; Plant Physiol 1994 104: 505-513; Cell 85 (1996) 171-182; Clouse et al. 1993 J. Plant Growth Regul. 12 61-66; Clouse and Sasse (1998) Annu. Rev. Plant Physiol. Plant Mol. Biol 49 427-451; Sasse, Steroidal Plant Hormones. Springer-

It is thus believed, without being bound to any theory, that modification of the RKS genes will result in a modification of the brassinosteroid pathway, thereby giving the various phenotypes that are shown below.

Verlag Tokyo pp 137-161 (1999).

20

25

30

35

15

"Functionally equivalent" as used herein is not only used to identify the functional equivalence of otherwise not so homologous genes encoding ELS, RKS, NDR/NHL or SBP/SPL proteins, but also means an equivalent gene or gene product of genes encoding ELS, RKS, NDR/NHL or SBP/SPL proteins in Arabidopsis Thaliana, e.g. identifying a homologue found in nature in other plants or a homologue comprising a deliberate nucleic acid modification, such as a deletion, truncation, insertion, or deliberate codon substitution which may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, and/or the amphipathic nature of the residues as long as the biological activity of the polypeptide is retained. Homology is generally over at least 50% of the full-length of the relevant sequence shown herein. As is well-understood, homology at the amino acid level is generally in terms of amino acid similarity or identity. Similarity allows for "conservative variation", i. e. substitution of one

hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Deliberate amino acid substitution may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, and/or the amphipathic nature of the residues as long as the biological activity of the polypeptide is retained. In a preferred embodiment, all percentage homologies referred to herein refer to percentage sequence identity, e.g. percent (%) amino acid sequence identity with respect to a particular reference sequence can be the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, without considering any conservative substitutions as part of the sequence identity. Amino acid similarity or identity can be determined by genetic programs known in the art.

10

15

20

25

30

35

'Plant cell', as used herein, amongst others comprises seeds, suspension cultures, embryos, meristematic regions, callous tissues, protoplasts, leaves, roots, shoots, bulbs, gametophytes, sporophytes, pollen and microspores. A target plant to be modified according to the invention may be selected from any monocotyledonous or dicotyledonous plant species, such as for example ornamental plants, vegetables, arable crops etc. 'Dicotyledoneae' form one of the two divisions of the flowering plants or angiospermae in which the embryo has two or more free or fused cotyledons. 'Monocotyledoneae' form one of the two divisions of the flowering plants or angiospermae in which the embryo has one cotyledon. 'Angiospermae' or flowering plants are seed plants characterized by flowers as specialized organs of plant reproduction and by carpels covering the ovaries. Also included are gymnospermae. Gymnospermae are seed plants characterized by strobili as specialized organs for plant reproduction and by naked sporophylls bearing the male or female reproductive organs, for example woody plants. 'Ornamental'

plants are plants that are primarily in cultivation for their habitus, special shape, (flower, foliage or otherwise) colour or other characteristics which contribute to human well being indoor as cut flowers or pot plants or outdoors in the man made landscape, for example bulbous plant species like Tulipa, Freesia, Narcissus, Hyacinthus etc. 'Vegetables' are plants that are purposely selected or bred for human consumption of foliage, tubers, stems, fruits, flowers or parts of them and that may need an intensive cultivation regime. 'Arable crops' are generally purposely bred or selected for human objectivity's (ranging from direct or indirect consumption, feed or industrial applications such as fibers) for example soybean, sunflower, corn, peanut, maize, wheat, cotton, safflower and rapeseed.

10

15

20

The invention provides a method for modulating a developmental pathway of a plant comprising modifying a gene encoding for a gene product or protein belonging to a developmental cascade or signaling complex comprising modifying at least one gene encoding a gene product belonging to the complex of RKS proteins, ELS proteins, NDR/NHL proteins, SBP/SPL proteins and ligand proteins. In one embodiment, the invention provides a method for modulating or modifying organ size. Plant or plant organ size is determined by both cell elongation and cell division rate. Modifying either one or both processes results in a change in final organ size. Increasing the level of 25 specific members of the family of RKS genes results in an increase in organ size, growth rate and yield. Modulating plant growth, organ size and yield of plant organs is the most important process to be optimized in plant performance. Here we show that modulating the level of members of the family of the RKS signaling complex with a method according to the 30 invention is sufficient to modulate these processes. The invention provides herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling 35 complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating

cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKS10 gene or functional equivalent thereof. Inactivation of endogenous RKS gene product results in a decrease in plant growth,

- 5 proving that the normal function of these endogenous RKS gene products is the regulation of growth and organ size. Use of a method according to invention for elevation of the levels of the regulating of the RKS signaling complex in plant cells is provided in order to increase for example the size of plant
- organs, the growth rate, the yield of harvested crop, the yield of total plant material or the total plant size. Decreasing the levels of endogenous RKS gene product is provided in order to decrease the size of plant organs, the growth rate, or the total plant size.
- In another embodiment, the invention relates to cell division. 15 The mitotic cell cycle in eukaryotes determines the total number of cells within the organism and the number of cells within individual organs. The links between cell proliferation, cell differentiation and cell-cycle machinery
- are of primary importance for eukaryotes, and regulation of these processes allows modifications during every single stage of development. Here we show that modulating the level of members of the family of the RKS signaling complex is sufficient to modulate these processes. The invention provides
 - herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and
- RKS/ELS ligand protein allowing modulating cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKS10 gene or functional equivalent Herewith the invention provides a method for modulating the number of cells to be formed within an 35

30

eukaryotic organism as a whole or for modulating the cell number within individual organs is, which of primary importance in modulating plant developmental processes,

especially of arable plants. Here we show that members of the RKS signaling complex are able to regulate the number of cellular divisions, thereby regulating the total number of cells within the organism or different organs.

5

10

15

20

25

In a further embodiment, the invention relates to the regeneration of apical meristem. Modification the levels of different RKS and ELS genes within plants allows the initiation and / or outgrowth of apical meristems, resulting in the formation of large numbers of plantlets from a single source. A number of gene products that is able to increase the regeneration potential of plants is known already. Examples of these are KNAT1, cycD3, CUC2 and IPT. Here we show that modulation of the endogenous levels of RKS genes results in the formation of new shoots and plantlets in different plant species like Nicotiana tabacum and Arabidopsis thaliana. Herewith the invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating apical meristem formation, in particular wherein said gene comprises an ELS1, RKS0, RKS3, RKS4, RKS8 or RKS10 gene or functional equivalent thereof. A direct application of such a method according to the invention is the stable or transient expression of RKS and ELS genes or gene products in order to initiate vegetative reproduction. Regeneration can be induced after overexpression of for example RKSO and ELS1; or by co-suppression of for example the endogenous RKS3, RKS4, RKS8 or RKS10 genes. Overexpression or co-suppression of these RKS and ELS gene products can be either transient, or stable by integration of the corresponding expression casettes in the plant genome. A further example of essentially identical functions for for example ELS1 and RKS0 overexpressing plants is for example shown in the detailed description, example 3, where both transgenic constructs are able to induce the

19 regeneration capacity of in vitro cultured Arabidopsis callus. Another example comprises functional interaction between RKS and SBP proteins which was shown by studies in transgenic tobacco plants in which SBP5 and RKS0 were both overexpressed under the control of an enhanced 35S promoter. At the tip of double overexpressing plants, embryostructures appeared whereas in the SBP5 overexpressing plants alone or the RKSO overexpressing plants alone no phenotype was detectable at the root tips of transgenic tobacco plants. These results show 10 that both RKS and SBP proteins are involved together in a signaling cascade, resulting in the reprogramming of developmental fate of a determined meristem. Furthermore, it is herein also shown that several RKS genes are able to regulate proper identity and development of 15 meristems and primordia. The invention for example also relates to fasciation, Fasciation is normally a result from an increased size of the apical meristem in apical plant organs. Modulation of the number of cells within the proliferating zone of the shoot apical meristem results in an excess number 20 of cellular divisions, giving rise to excess numbers of primordia formed or to stems in which the number of cells is increased. The invention herewith provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said 25 gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating fasciation, in particular wherein said gene comprises an RKSO, RKS3, RKS8 or RKS10 gene or functional 30 equivalent thereof. Here we for example show that modulation of the levels of RKS gene products in plants like Arabidopsis thaliana can result in fasciated stems. A direct application as provided herein is the regulated formation of fasciation in plant species in which such a trait is desired like 35 ornamental plants. Regulation of the initiation and extent of fasciation, either by placing the responsible RKS encoding DNA sequences under the control of stage or tissue specific

promoters, constitutive promoters or inducible promoters results in plants with localized or consitutive fasciation of stem tissue. Another application is modulating the number of primordia by regulation of the process of fasciation. An example is provided by for example sprouts, in which an increased number of primordia will result in an increased numbers of sprouts to be harvested. Fasciation can also result in a strong modification in the structural architecture of the inflorescence, resulting in a terminal group of flowers resembling the Umbelliferae type.

10

15

20

25

30

35

Identical phenotypes can be observed when transgenic plants are produced that contain the NHL10 cDNA under control of an enhanced 35S promoter. The resulting phenotype of the resulting flowers show that flower organ primordia are switched in identity, similar as observed for RKS10 and RKS13. These meristematic identity switches are normally never observed in Arabidopsis and the fact that two different classes of genes are able to display the same phenotypes in transgenic plants is a clear indication for a process in which both members of the RKS and the NDR/NHL families are involved. The invention also relates to root development. Fasciation is normally a result from an increased size of the apical meristem in apical plant organs. Modulation of the number of cells within the proliferating zone of the root apical meristem results in an excess number of cellular divisions, giving rise to excess numbers of primordia formed or to roots in which the number of cells is increased. Adaptation to soil conditions is possible by regulation of root development of plants. Here we describe several processes in root development that can me manipulated by modification of the levels of RKS signaling complex within the root. The invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating root development, in particular

wherein said gene comprises an ELS1, ELS2, RKS1, RKS3, RKS4, RKS6, RKS8 or RKS10 gene or functional equivalent thereof. Root length, a result by either root cells proliferation or elongation, can for example be increased by overexpression of 5 for example RKS3, RKS4, RKS6 and ELS2, or inactivation of the endogenous RKS10 gene product. Root length can also be decreased by decreasing of endogenous RKS1 levels or by strong overexpression of RKS10. The initiation of lateral roots is also regulated by RKS gene products. Overexpression of for example RKS10 can result in a strong increase in the 10 initiation and outgrowth of lateral roots. Co-suppression of RKS1 also resulted in the initiation and outgrowth of large numbers of lateral roots. Root hair formation and elongation is important in determining the total contact surface between plant and soil. A strong increase of root hair length 15 (elongation) can be obtained by overexpression of ELS1 and RKS3 gene products. As the roots of terrestrial plants are involved in the acquisition of water and nutrients, anchorage of the plant, synthesis of plant hormones, interaction with the rhizosphere and storage functions, increasing or 20 decreasing root length, for example for flexible adaptations to different water levels, can be manipulated by overexpressing or cosuppressing RKS and / or ELS gene products. Modulation of the total contact surface between plant cells and the outside environment can be manipulated by regulation lateral root formation (increased by RKS10 overexpression and co-suppression of RKS1). Finally the contact surface between plant cells and the soil can be influenced by modulation of the number of root hairs formed or the elongation of the root hairs, as mediated by ELS1 and 30 RKS3.

In a further embodiment, the invention relates to apical meristem identity. All parts of the plant above the ground are generally the result on one apical shoot meristem that has been initiated early at embryogenesis and that gives rise to all apical organs. This development of a single meristem into complex tissue and repeated patterns is the result of tissue

and stage-dependent differentiation processes within the meristems and its resulting offspring cells. The control of meristem formation, meristem identity and meristem differentiation is therefore an important tool in regulating plant architecture and development. Here we present evidence the function of RKS and ELS gene products in regulation of the meristem identity and the formation and outgrowth of new apical meristems. The invention provides a method for modulating a developmental pathway of a plant or plant cell 10 comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating meristem identity, in particular wherein said gene comprises an ELS1, RKS8, RKS10 or RKS13 gene or functional 15 equivalent thereof. Introduction of for example the RKS10 gene product or an other member of the RKS signaling complex under the control of a tissue and / or stage specific promoter as provided herein allows localized and time regulated increases 20 in the levels of gene product. For example the meristematic identity in a determined meristem might thereby be switched back into an undetermined meristem, thereby changing for example a terminal flower into an undetermined generative meristem.

Another application might be found in changing the meristematic identity at an early time point, during early vegetative growth, thereby switching the vegetative meristem into a generative meristem, allowing early flowering.

Modulation of meristem identity in terminal primordia, like for example as shown in Figure 30, where flower organ primordia are converted into terminal flower primordia, allows the formation of completely new types of flowers and fused fruitstructures. Constitutive overexpression of RKS gene products results in plants with many apical meristems, as can clearly been seen in Figure 29, where RKS10 overexpression results in an extremely bushy phenotype.

In another embodiment, the invention relates to male sterility. Male sterility is a highly desired trait in many plant species. For example, manipulation of pollen development is crucial for F1 hybrid seed production, to reduce labour costs and for the production of low-environmental impact genetically engineered crops. In order to produce hybrid seed from inbred plant lines, the male organs are removed from each flower, and pollen from another parent is applied manually to

with a number of vegetables (e.g. hybrid tomatoes) and with many ornamental plants. Transgenic approaches, in which one or more

produce the hybrid seed. This labour-intensive method is used

introduced gene products interfere with normal pollen initiation and development is therefore highly desired.

15 Especially when the number of revertants (growing normal pollen) is extremely low.

20

25

Male sterility in plants is a desired trait that has been shown already in many plant species as a result of the inactivation of expression of a number of genes essential for proper stamen development, mitotic divisions in the pollen stem cells, or male gametogenesis. A method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling

complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating pollen development, in particular wherein said gene comprises an ELS2 or RKS10 gene or functional equivalent thereof.

Here we present data that show that overexpression of gene products, like transmembrane receptor kinases (RKS) and extracellular proteins (ELS) can also result in the formation of male sterility. The ability to induce male sterility by overexpressing specific genes as provided herein allows the opportunity to produce transgenic overexpressing plants in which the pollen development is inhibited. Stable single copy homozygous integration of such overexpressing traits into the

plant genome will render such plants completely sterile, making them excellent material for the production of F1 hybrid seed. Furthermore, the combined integration of a male sterility inducing overexpressing gene coupled directly with another desired transgene result in transgenic plants which are unable to produce transgenic seed, making these transgenic plants excellent material for outside growth without problems affecting transgenic pollen spreading throughout the environment, thereby eliminating possible crosses 10 with wild plant species or other non-transgenic crops. The combination of a desired transgene flanked on both sites by different male-sterility inducing overexpressing genes would decrease the frequency of pollen formation to an extremely low level. An example is an overexpressing construct of RKS10 15 at the 5'end of integrated DNA fragment, the desired transgene expression cassette in the middle and at the 3'end of the integrated DNA the ELS2 overexpressing construct. This complete DNA fragment is integrated into the genome by conventional techniques, like particle bombardment, 20 Agrobacterium transformation etc. Another possible application concerns the modification of pollen in ornamental plant species like lily, where the release of pollen from cut flowers can be avoided by making transgenic plants in which pollen development is initiated by release from the stamen is 25 prevented (a desired trait that can be obtained by overexpressing for example ELS2, resulting in partial pollen development). Hereby the ornamental value of the stamen with pollen is not lost, but release of pollen is inhibited.

Furthermore, surprisingly we observe that NDR NHL gene products share homology with the family of syntaxins, involved in vesicle transport, positioning of cell wall formation and cytokinesis.

Table 1

Homology between members of the syntaxin family and the NDR NHL family

5 NHL10= At2g35980

maaeqplnga fygpsvpppa pkgyyrrghg rgcgccllsl fvkviisliv ilgvaalifw livrpraikf hvtdasltrf dhtspdnilr ynlaltvpvr npnkriglyy drieahayye gkrfstitlt pfyqghkntt vltptfqgqn lvifnagqsr tlnaerisgv ynieikfrlr vrfklgdlkf rrikpkvdcd dlrlplstsn gttttstvfp ikcdfdf

10

At1g32270 syntaxin,

MVRSNDVKFQ VYDAELTHFD LESNNNLQYS LSLNLSIRNS KSSIGIHYDR FEATVYYMNQ RLGAVPMPLF YLGSKNTMLL RALFEGQTLV LLKGNERKKF EDDQKTGVYR IDVKLSINFR VMVLHLVTWP MKPVVRCHLK IPLALGSSNS TGGHKKMLLI GQLVKDTSAN LREASETDHR RDVAQSKKIA DAKLAKDFEA ALKEFQKAQH ITVERETSYI PFDPKGSFSS SEVDIGYDRS QEQRVLMESR RQEIVLLDNE ISLNEARIEA REQGIQEVKH QISEVMEMFK DLAVMVDHQG TIDDIDEKID NLRSAAAQGK SHLVKASNTQ GSNSSLLFSC SLLLFFFLSG DLCRCVCVGS ENPRLNPTRR KAWCEEEDEE QRKKQQKKKT MSEKRREEK KVNKPNGFVF CVLGHK*

20

15

Below the homology is shown between NHL10 (Upper line) and a syntaxin protein. (bottom line). The identical amino acids are shown in the middle line.

25

IVRPRAIKFHVTDASLTRFDHTSPDNILRYNLALTVPVRNPNKRIGLYYDRIEAHAYYEG
VR KF V DA LT FD S N L Y L RN IG YDR EA YY
MVRSNDVKFQVYDAELTHFDLESNNN-LQYSLSLNLSIRNSKSSIGIHYDRFEATVYYMN

30

KRFSTITLTPFYQGHKNTTVLTPTFQGQNLVIFNAGQSRTLNAERISGVYNIEIKFRLRV
R FY G KNT L F GQ LV GVY I K
QRLGAVPMPLFYLGSKNTMLLRALFEGQTLVLLKGNERKKFEDDQKTGVYRIDVKLSINF

35 RFKLGDLKFRRIKPKVDCDDLRLPLSTSNGTTT

R L KP V C L PL T

WO 2004/007712 PCT/NL2003/000524 26

That syntaxins and NDR/NHL genes share large homology becomes even more clear when performing a database search using the following site:

http://mips.gsf.de/proj/thal/db/search/search frame.html

5 searching for homologous sequences with the sequence At1g32270

gene Code:

predicted function:

	At1g32270	syntaxin, putative	Syntaxin	
10	At5g46860	syntaxin related protein	Syntaxin	
	AtVam3p (g	gb AAC49823.1)		
	At4g17730	syntaxin	Syntaxin	
	At5g16830	syntaxin homologue	Syntaxin	
	At3g11650	unknown protein	Putative	syntaxin
15	At2g35460	similar to harpin-induced protein	Putative	syntaxin
	At5g06320	harpin-induced protein-like	Putative	syntaxin
	At2g35980	similar to harpin-induced protein	Putative	syntaxin
	At1g65690	hypothetical protein	NDR HNL	
	At4g05220	putative protein	Putative	syntaxin
20	At3g05710	putative syntaxin protein	Syntaxin	
	AtSNAP33			
	At2g27080	unknown protein	NDR HNL	
	At3g52470	putative protein	Putative	syntaxin
	At1g61760	hypothetical protein	Putative	syntaxin
25	At5g21130	putative protein	NDR HNL	
	At3g52400	syntaxin-like protein synt4	Syntaxin	
	At2g35960	putative harpin-induced protein	Putative	syntaxin
	At5g06330	harpin-induced protein-like	Putative	syntaxin
	At5g26980	tsnare	Syntaxin	
30	At5g36970	putative protein	Putative	syntaxin
	At3g44220	putative protein	Putative	syntaxin
	At3g03800	s-syntaxin-like protein	Syntaxin	
	At2g35970	putative harpin-induced protein	Putative	syntaxin
	At4g09590	putative protein	Putative	syntaxin
35	At4g23930	putative protein	•	
	At1g61290	similar to syntaxin-related protein	Syntaxin	
	At3g11660	unknown protein	Putative	syntaxin
	At1g54540	hypothetical protein	Putative	syntaxin
	At3g24350	syntaxin-like protein	Syntaxin	
40	At5g22200	NDR1/HIN1-like	NDR HNL	

	WO 2004/007712	PCT/NL2003/000524			
	At1g11250 syntaxin-related protein At-SYR1	Syntaxin			
	At5g53880				
	At3g11820 putative syntaxin	Syntaxin			
	At3g54200	Putative syntaxin			
5	At5g05760 t-SNARE SED5	Syntaxin			
	At5g53730	Putative syntaxin			
	At4g03330 SYR1-like syntaxin 1	Syntaxin			
	At3g47910				
	At5g08080 syntaxin-like protein	Syntaxin			
10	At5g11890	Putative syntaxin			
	At1g17620	Putative syntaxin			
	At2g22180	Putative syntaxin			
	At5g22870	Putative syntaxin			
	At2g46300	Putative syntaxin			
15	At2g27260	Putative syntaxin			
	At4g01410	Putative syntaxin			
	At5g22200	Putative syntaxin			
	At4g01110	Putative syntaxin			
	At3g52460	Putative syntaxin			
20	At3g26350	Putative syntaxin			
	At1g08160	Putative syntaxin			
	At2g01080 .	Putative syntaxin			
	At5g56050	Putative syntaxin			
	At3g20600	Putative syntaxin			
25	At3g20590	Putative syntaxin			
	At4g39740	Putative syntaxin			
	At1g32270	Putative syntaxin			
	At1g13050	Putative syntaxin			
00	At5g45320	Putative syntaxin			
30	At3g20610	Putative syntaxin			
	At4g26490	Putative syntaxin			
	At5942860	Putative syntaxin			

This observation provides the explanation for understanding 40 the mechanism by which the RKS / NDR-NHL complex functions.

Cell wall immobilized RKS gene products (containing the

Putative syntaxin

Putative syntaxin

At1g45688

At4g26820

WO 2004/007712 PCT/NL2003/000524
28
automain like automain respond to a local ligand

extensin-like extracellular domain) respond to a local ligand signal, in combination with the heterodimerizing ELS protein (s) either as homodimers, as RKS heterodimers or in combination with the heterodimerizing ELS protein(s).

5

15

Predicted ligands for the RKS / ELS receptor binding consist of peptide ligands (based on the LRR ligand binding domain of this class of receptors). These ligands are normally produced as a pre pro protein. The N-terminal signal sequence is removed by the transport through the Golgi system and allows modification of the ligand at this stage (e.g. glycosylation). The ligands can then be secreted after which further processing is possible (e.c. proteolytic cleavage, removal of sugar groups etc.) The resulting peptide, possible as a monomer or a (hetero)dimerizing molecule binds the transmembrane receptor complex with high affinity, resulting in transmission of the signal from the ligand through the transmembrane receptor component towards the other site of the membrane.

One class of ligands interacting with the RKS and / or ELS receptors consists of the family of pre(pro)proteins shown hereunder in table 3.

Table 3 Ligands within the RKS signaling complex (herein also called RKS/ELS ligand proteins)

For each ligand (A to N) the genomic structure before splicing and processing 5'- towards 3' is given. Exons are indicated in large letters; introns and surrounding sequences (including leader 5'-and trailer sequences 3'-) are indicated in small letters.

Beneath each DNA sequence the amino acid sequence of the pre-propeptide is given. The first line represents the signal sequence

The second (set of) lines represents the pro-peptide.

The last line represents the conserved Cysteine motif.

A. At1g22690

15 1 attaaacqcc aaacactaca tctgtgtttt cgaacaatat tgcgtctgcg tttccttcat 61 ctatctctct cagtgtcaca atgtctgaac taagagacag ctgtaaacta tcattaagac 121 ataaactacc aaaqtatcaa qctaatgtaa aaattactct catttccacg taacaaattg 181 agttagetta agatattagt gaaactaggt ttgaatttte ttettettet tecatgeate 241 ctccgaaaaa agggaaccaa tcaaaactgt ttgcatatca aactccaaca ctttacagca 20 301 aatgcaatct ataatctgtg atttatccaa taaaaacctg tgatttatgt ttggctccag 361 cgatgaaagt ctatgcatgt gatctctatc caacatgagt aattgttcag aaaataaaaa 421 gtagctgaaa tgtatctata taaagaatca tccacaagta ctattttcac acactacttc 481 aaaatcacta ctcaagaaat ATGAAGAAGA TGAATGTGGT GGCTTTTGTT ACGCTGATCA 25 541 TCTCTTTTCT TCTGCTTTCT CAGgtaaact gttaaaacca ttttcaagac taccttttct 601 ctatttcaga caaaccaaag taaaacaatg aaaaatctct ctggtctttc atagGTACTT 661 GCAGAGTTGT CATCATCCAG CAACAATGAA ACTTCCTCTG TTTCTCAGgt aagagtgata 721 caaaaacata ctaaacaaac tttcaagaga gtaatatata aggaaatgtt ggcttctttt 781 ttttuttuct aatcayACGA ATGACGAGAA CCAAACTGCG GCGTTTAAGA GAACATACCA 30 841 CCATCGTCCA AGAATCAgtt agtctactct ttcaacactc taattccttt gttctaagta 901 tttttttgc ccccacaac cttttttta ttaaatgagc caatttttat agATTGTGGG 961 CATGCATGCG CAAGGAGATG CAGTAAGACA TCGAGGAAGA AAGTTTGTCA CAGAGCCTGT 1021 GGAAGTTGTT GTGCCAAGTG TCAGTGTGTG CCGCCGGGAA CCTCCGGCAA CACAGCATCA 1081 TGTCCTTGCT ACGCCAGTAT CCGTACACAT GGCAATAAAC TCAAATGTCC TTAAaagact 35 1141 teteatttet caactatagt eteatettet gattatgttt ettettttgt tatgttgcat 1201 gtgtgatgtg tgagcttatt attatgttga ttgttgacat aattcaacta tataatttgt 1261 atcgattccg aataataaga tgagtgattt tattggctat taagtttttt ttttttttt 1321 ttgggcacaa tggctattaa gttttaaaca tetgatttta ttggttacaa aaaacaacaa 1381 agtttcattt tcatattaac acaaaatctc catacatatt accaaaccaa aaaaatacac 1441 aagggggaga gagaccaacg gttcttggtt cagagtttgc atcttgtttg agccgtcacc 1501 gtttcttaga cttaacagcc acaacacctt tataaagctt cacgcgatcc ttcaacgcat 40 1561 ctcgccgagg ccgagccacc ttattgtttg gatcaaacaa caaaacttct tcaaacgcat 1621 tcaatgccaa aggc

45 MKKMNVVAFVTLIISFLLLSQVLA

50

ELSSSSNNETSSVSQTNDENQTAAFKRTYHHRPRIN

 ${\tt CGHACARRCSKTSRKKVCHRACGSCCAKCQCVPPGTSGNTASCPCYASIRTHGNKLKCP*}$

B. At1g74670

	1	gaaaaaaaga	agaaaagata	atggtccgta	ttaatatagt	tgaaaacttg	aaactacttt
5	61	ttagtttgta	tataatacag	tagactaggg	atccagttga	gtttctttct	ttattttgag
	121	tttgtgttta	tgtttgattt	tacgttttta	tatgtaaata	agatatttta	cgaattatgg
	181	ttttatttgg	gtagaagttg	tagaatgact	taaacaatca	agtggcagaa	tgagatatat
	241	aaagtaatat	aatatatgta	ccgttattaa	cttattgtac	atgtgaatga	ggaagcttac
		acacacacac					
10		atctctcaaa					
		teteteaaga					
		TCACTTTCGT					
		tttattttt					
		tccctttcta					
15		cagTATGGAC					
		atcttgtttc					
		GACAATGCAC					
		AAAAGTGTTG					
		GTCCTTGTTA					
20		cattyagaga					
		gttgtcgtga					
		atcatataaa					
		gaaatgtgta					
		ctttcgtata					
25		attttacttt					
		tttagtataa					
		ctatatcaat		aatctgcgct	tccttctagt	tttttacaat	tatggagatt
	1441	tttcgacgat	gat				

30

MAKLITSFLLLTILFTFVCLTMS

KEAEYHPESYGPGSLKSYQ

35

 $\texttt{CGGQCTRRCSNTKYHKPCMFFCQKCCAKCLCVPPGTYGNKQVCPCYNNWKTQQGGPKCP} \ \ *$

C. At1g75750

	1	cacaactttt	atacgcacca	ccaaccgacc	cattttgaaa	aagagaaaat	aaaccacaaa
5	61	aacacacata	aataatatgc	tgataacaat	gtcttaaaaa	tctatttacc	atttctagta
	121	atcaatatct	attgcaaaaa	atatttataa	gaatacaaat	gaaaaatgat	aaaatacaaa
	181	tgatttctca	attacctaaa	aaatataaaa	atgtcttact	ttattttcag	ccactgttgg
			caatcatatc				
			aacttttaat				
10			tgtccatcca				
			taaaaccaca				
			ttggagaatc				
			TCTCCAACTC				
			tataatactt				
15			AAACTCACAG				
			acctaacgtt				
			ATTGTGGGAG				
			GAGCGTGCGG				
~~			ACGACAAGTG				
20			AAgaagaaac				
			tactttggcg				
			gtgagtactg				
			tttgttttt				
~~			aagattatgt				
25			ggaaaagtat				
			tagactagct				
			taccattatt			taattcttgt	ttgcgttata
	1441	atttgtgtta	atacgataga	gtagacaaat	ga		

30

 ${\tt MAISKALIASLLISLLVLQLVQA}$

DVENSQKKNGYAKKID

35

 ${\tt CGSACVARCRLSRRPRLCHRACGTCCYRCNCVPPGTYGNYDKCQCYASLTTHGGRRKCP*}$

D. At2g14900

```
1 ataactaaca atggttgagt ggagatgtgc ttttagtcaa gtggttaaat atatttgact
 5
            61 togttttttt cattggagtt tgactctact aagttgtgtt toctcgcgta gtaagaattg
           121 gttatggatt agaccgtate gatetaaaga tgtcaaagaa aaaaaaaatgt ggttgtgtaa
           181 agtaaatatg tagattgtgg cggattaaag tatgttttga ttcacatcat tattgttatt
           241 ttttcatgaa ttctaaatgt aaagttctta taatcttatg ttacttttta caaattgtaa
           301 ggattactct gaaatttggt atcgaattct aagacaaata caaaataaca atgactgaac
10
           361 aagttgataa aacataatgg aaggaataat actgcagttc tattaaatac taaagaagtt
          421 ggtagattgg cctataaaag gagaataaag agaccacaag aaggtetatt atteggggac 481 taaagaaage caaagaaaac ATGAAAATAA TAGTETECAT CTTAGTGTTA GCCTCTCTTC
           541 TTCTAATCAG TTCATCTCTT GCTTCGGCTA CTATATCAGg ttggttctaa tctcttcaag
           15
           661 gtcacaattg tttctttatg ctttcgtttc cataagaaaa atattacaaa tattaactag
           721 aacaacataa catgcaaacg agtaatacaa aattcattat tatgatcaaa acaatcatga
           781 attagttgga cttatttgtt aaattccgaa aatctcacta aaataaagtg aacttcatct
           841 acatggettt agacgcaaaa tetttaaggg tatetacaca agtttggaat gaataattte
           901 ttgcgatggt agtgtagaag gatctagaag atccacaaga tcattagtgt atcttctaga
20
          961 teettttaca ttgagaagtg aggagatatt tgttgtatta gaaagaatta tagtgaagta
          1021 aattittaa ctatgtacga tcatttatat acgatactit tattaaggat cttgtggatc
          1081 ttctagatgc TTTTGGTAGT GGCGCGGTAG CTCCGGCACC GCAGAGCAAA GATGGACCGG
          1141 CGTTAGAGAA ATGGTGTGGA CAGAAATGTG AAGGGAGATG CAAAGAAGCG GGGATGAAAG
          1201 ATCGGTGTTT GAAGTATTGT GGGATATGTT GCAAAGACTG TCAGTGTGTT CCTTCAGGCA
25
          1261 CTTATGGGAA TAAGCATGAA TGTGCTTGCT ATCGTGACAA GCTCAGTAGC AAAGGCACTC
          1321 CTAAATGTCC TTGAttctat ttctttccaa ccaaaaattt aaataaatga ataagagaga
          1381 tocagtaaac taatataaaa ctataaatgg atcttttgtt tatgattttt tttttttcat
          1441 ttctatttt acgaatttgt cttggtcttt ttgaagtaag tttttaaata ttgaaaagtg
          1501 ctaaaattat gtggaaatcg ataatgttaa tgaatgatat aatatataag tootcagttt
30
          1561 ttgtaagaaa cttgaatata aataatattt catcaaacat aataaataaa tatattgtat
          1621 aattagattg gctcaaccga tataaacaat tgaatcgaat tttttcttct aaatatttaa
          1681 tcatccaaat ttgtattgta ccaatgaatg agatggttat gaggactaga agatagagag
          1741 gagaagaacg tgtttggtaa aataattatg atggagttga gacaactttt aagagatttt
          1801 aaaaagactg actaacgtgt taggttcatc acgt
35
```

MKIIVSILVLASLLLISSSLASATIS

DAFGSGAVAPAPQSKDGPALEKW

40

CGQKCEGRCKEAGMKDRCLKYCGICCKDCQCVPSGTYGNKHECACYRDKLSSKGTPKCP*

E. At2g18420

1 gccaatgggt aactgaggaa gaaggataag accaaaaaaa aaactaaaat ggacagattg 5 61 aattagtaaa aagataaatt ctaaaaaccg aaacaaatct taagttggtg tatatacatc 121 tgcattgacc aacaaaagaa agtagactga aatttatttg aaaatgatct tgtaaaggca 181 tattatatat ttaatttagg aaatgaatgt taaatcettt aaattgtttt gattteacaa 241 aaggataaag aaatattggt tacatacatc ttaatgtgtt gaccaaaaca aataaaatgt 301 gataagaaac aataaaacca ttttgaccaa agttcttata gttttaatat tctttaattg 10 361 tcatttgtta gtgactaata atattacatt aaacctaatg tataaataga agccccatct 421 totacgoott tataattago aacaaccaaa aacattoatt tgtoattttg totootottt 481 tgttttetet gateactagt ATGGCTGTAT TCAGAGTCTT GCTTGCTTCT CTTCTCATAT 541 CTCTTCTTGT CCTCGACTTC GTCCATGCCG ATATGGTGgt acaattttaa caaccaaata 601 tattttctta tttgatttta ttttttcaca acttttgtct acgttctaat ggaatttttt 15 661 tcaaaatatt catgcagACG TCGAATGACG CCCCTAAAAT CGgtaatatc tctatcatat 721 aaacacgtac gttgaatttc tatatacgtg tgtttaattg aagttttggt tggaaattgt 781 atgtatttgt agattgcaac agcaggtgcc aagagCGGTG CAGTCTTTCG AGTAGGCCAA 841 ATCTTTGTCA CAGAGCGTGC GGGACTTGCT GCGCTAGGTG CAACTGCGTG GCACCGGGCA 901 CATCCGGAAA CTACGACAAA TGTCCGTGCT ATGGTAGCCT AACCACCCAC GGAGGACGCA 20 1021 ttatgagagt aattgtggtt attttcttgg gaattattaa aaagcaaaag aaagagaatg 1081 ttatacgtca tgtgcaactc ttcgatcttt gttttagtgt ttatccaatt tgtacttgtt 1141 ggtttggttc ctggttaaca ttaggtctga aaaggtattg tttttcatta tacaattcac 1201 taaataggca tegtaettge atataaaata aagaatgaag agagaagtaa aagagtttte 25 1261 tttttttact catggaagtt aggcaatggg tttaaatatg gtaacaacag aattggaggg 1321 gacttaatga actatgacgt aaaactgaga gcgattgaat atgtaacgtt accaacaata 1381 ccaataaaat tatgaaagat agtatatgaa attacgttta attaatgttt ccgggttgaa 1441 tgtattatat atagaagtaa cagtacgatt tttattacat ttttgtacaa gattcctaga 1501 aaggtataac ctctataaag ttaataatag tcttgagtct tgactcttcg aggcaaataa 30 1561 attcaccyca taattaatcy ttcaactatt attctatatt ctatataaca tyaycttcaa 1621 caaaagaage atcaatcata tetteaacag tataetgeag tgtaatgtaa catatteaag 1681 atcaaaccgg acaaaaaagc aagataccgt cgaaacaatc aaaccccatg tatcataaac 1741 toccatotto totttoctaa attoccogto gottgoacaa to

35 mavfryllasilislividfyha

DMVTSNDAPKID

40

CNSRCQERCSLSSRPNLCHRACGTCCARCNCVAPGTSGNYDKCPCYGSLTTHGGRRKCP*

WO 2004/007712 PCT/NL2003/000524 34

F. At2g30810

-	1	cttttatttg	tttgtgaaaa	aaaacaatag	cttttatttg	tcctaggaat	tatttaatag
5	61	attaaataac	agctattttt	ctcttatttc	ttagtgatta	aaatatttaa	aatacagacc
	121	aaaattaatt	gtttatgtta	atatatttac	tccttaatcc	tttatattaa	aattgtataa
	181	tgcatgtagt	taataaattg	ttttccaaaa	ttcattcata	attttattcc	taaattattt
	241	tggtcaagaa	aacacatctt	tgaataatta	aatgcttcct	tgtatttgat	aatttcttga
4.0	301	tattttaaaa	taccttctat	actatgccaa	tgttattggt	tataaatagg	tttaacatto
10	361	atcctgaaat	atatcataag	aaaatcaaaa	gtgaaataag	agatcaaaAT	GATGAAGCTC
	421	ATAGTTGTCT	TTGTTATATC	CAGTTTGTTG	TTTGCTACTC	AATTTTCTAA	Tgtaaaaatt
	481	attattattt	tcttcatatt	atgatttatg	aattcagaga	aataaagttt	tttttttat
	541	gtgtgtatgt	acagGGTGAT	GAATTAGAGA	GTCAAGCTCA	AGCACCTGCA	ATCCATAAGg
4 ==	601	tatatttaaa	ttataaaata	tcaaatactg	aataataaat	aataaatata	ttacaacaag
15	661	aatatcaatg	ttattttca	aactacataa	ttttaaaata	ttttattgat	aacacaaato
	721	tatattatta	tcgtctccat	tgatttgcat	tctaaatttg	tttttgttat	ccaaccaatt
	781	tcagAATGGA	GGAGAAGGCT	CACTTAAACC	AGAAGgtaaa	ttgtttaaaa	gatattattt
	841	ttatttatat	agtaaatgat	tgatcaaatc	acaacttaaa	taatttaatt	gttgatttat
						TTCGGCGACA	
20	961	AACCATGTTT	GTTTTTTTGC	AACAAATGTT	GTAACAAATG	TTTGTGTGTA	CCATCGGGAA
	1021	CATATGGACA	CAAAGAAGAA	TGTCCTTGCT	ACAATAATTG	GACGACCAAA	GAAGGTGGAC
	1081	CAAAATGTCC	ATGAaaacaa	aaaattgtaa	aagcaaaata	aaatctatcg	ttgttatctc
	1141	tcaataaaat	ctatgtttgt	aatccttgtt	tttcaatata	gaatataata	tggagttttc
~=	1201	ataatttctt	ctattacaaa	attaaagtta	atgcacaaat	aaattgaagg	gacttggace
25	1261	ttttcgtgta	agttctttct	ttaaatcacg	aacaatttag	atttatattt	tcactcttac
	1321	aaacacaaaa	catggatgct	ctttaactct	catccaaaca	aaatgcattt	ctctctttct
						agatatatga	
	1441	ttgaatttat	ttaggccatg	ttttaaaatc	gtgtttggtt	agattgaccc	atgaaatgtt
00	1501	gacatatttt	aacattccta	aatatgacta	aaaatgatta	aagatattta	ataatatatt
30			aaaatgatta				

MMKLIVVFVISSLLFATQFSNG

35

DELESQAQAPAIHKNGGEGSLKPEE

 ${\tt CPKACEYRCSATSHRKPCLFFCNKCCNKCLCVPSGTYGHKEECPCYNNWTTKEGGPKCP*}$

WO 2004/007712 PCT/NL2003/000524 35

G. At2g39540

1 taatgctata cttttaatct ataatatata ttagatgtga cttaaggaat ttcaatagtt 61 atacataata ataaaaatga atattigtta gigttacaaa cigigigica taatcatcat 5 121 tcatcaggat ttcaaaaata tctcaaaatt gttgtaagtt catgtaattc gaaatgaatg 181 tgcactataa gaaataaatt tacaatttaa aaaatgcttc aatactggtt acaaaaaaaaa 241 ctttcaatac tagtattata ctacttactt agtcaaaaaa gtttatgaat atggttttt 301 ctgtatgtta atatttttaa ctgaaaatag taccgacata acaagtaaag atatctttat 361 ttaaagtaac aaacattaat ttcacttcaa attctcacta ttaaggattc ctctctttgt 10 421 agccacattt caccatcact actttgtttt cgcatatctt taaattttgt atacgtagca 481 aactettteg agaaaacaag ATGAAGCTCG TGGTTGTACA ATTCTTCATA ATCTCTCTTC 541 TCCTCACATC TTCATTTTCT GTACTTTCAA GTGCTGATTC GTgtaagtgt ttacttaatc 601 tagttaataa ttgtaggtca tgcatgtatc attttgaaac aagttttctg aaattctaag 661 attttacata tatatgtgat aaatgaatta gcagCATGCG GTGGAAAGTG CAATGTGAGA 15 721 TGCTCAAAGG CAGGACAACA TGAAGAATGC CTCAAGTACT GCAATATATG TTGCCAGAAG 781 TGTAATTGTG TTCCTTCGGG AACTTTTGGA CACAAAGATG AATGTCCTTG CTACCGTGAT 841 ATGAAAACT CCAAAGGTGG ATCCAAGTGT CCTTGAacgt tctttgaaga tcctcatcac 901 atacatataa cttctacgta ctatatgtgt ggaaatatta atcacattct atgtttgaaa 961 tatataaaat aaaatcaatg cccccaatgt tggaaatctt caatgtgata tcttaatata 20 1021 tatcacgaat aaaaaagttt aaatttetea ateteatttt taatetttaa tetaatttet 1081 taacacatca acgaatcttt aatctttaat catgtagata attatcagag cacctaaaca 1141 ttgcgccgtt ttgtgattat acaaagtaac atcgtgctgt ttttgacttt tgaaaaccac 1201 agatccaaaa actgtttact ttcctctaag agaaagcaaa gccgagtgag tccaagcgag 1261 ttttgagaga ttcgttgact cactaccgga gaacgacgct atgtcagaga ccgccgtgtc 25 1321 aatogattog gaccgatota agtoggagga agaagacgaa gaagagtatt otocac

MKLVVVQFFIISLLLTSSFSVLSSA

30

35

DSS

CGGKCNVRCSKAGQHEECLKYCNICCQKCNCVPSGTFGHKDECPCYRDMKNSKGGSKCP*

WO 2004/007712

PCT/NL2003/000524

H. At3g02885 (GASA5)

5 1 cgctttctat tacacttttt tttcttttta gtcgcacttc acaattagct taattaattt 61 cctaaactcg cttattttcc cctttctata tacagatatt atcattagtg acattttcat 121 tttccaaaca gagcgtttag acactagtca actacacaat ataattttcc aattttcact 181 gagagaaatg ttttttttt ttttttccaa ggcaagattt tagtcttttg gttctctata 241 cgtgggtaat tagtgattag taatttacac tgttgagtct ttgacattgt ctaagagaca 10 301 aaaacgacaa gtgtggtacg taattagaaa ttaaaatgac ctacttcccc agaatcacgg 361 catgaacatt ygcaatacca aatttettga ataccattga aggaaateca cactaatcat 421 tttctctata aatatcttta atccgtttta ttgtttctta agaatcattc attggcaatc 481 aagatttttt aaccaaaaaa ATGGCGAATT GTATCAGAAG AAATGCTCTT TTCTTCTTGA 541 CTCTTCTCTT TTTATTGTCA GTCTCCAACC TCGTTCAGgt aaaccactca aaacagattc 15 601 agtttattaa agtctgatat tgaagtttta tatattacag gctgctcgtg gaggtaaaaa 661 tgaccaaagg ctatacattc cttaaaaatt taatggctat tagttttctg atattgaagt 721 tttatatata tatgacagGC TGCTCGTGGT GGTGGCAAAC TCAAACCCCA ACgtacggac 781 tcaaaacttt tgttgtttca tatgatcata ttaatttatt aatcactaat tattgataat 841 gttgataaat aaactttaaa gtaacaataa tggtgtttat tttgtgaaat gtcagttttc 20 901 tagtatactg tatgctgtga attataagca tgaacataaa gatctcaatg atttgtttt 961 tgtttgtttg ttgtgatatg cttttttgat ggaaacttca attgtagAGT GCAACTCAAA 1021 GTGTAGCTTC CGTTGTTCAG CAACATCACA CAAGAAGCCA TGCATGTTCT TTTGCCTCAA 1081 GTGTTGCAAA AAATGTCTTT GTGTTCCTCC TGGCACTTTC GGCAACAAAC AAACTTGTCC 1141 ATGTTACAAC AACTGGAAGA CTAAAGAAGG CCGTCCAAAA TGTCCTTAAa acttctttt 1201 agatatattt gataatattc atctagtttt ggattatcaa acacttacta ctctgtttta 25 1261 atctgtttct acaagttggc gatttgtctc tacacttttt ttgtgtcttt tgctcttaac 1321 tgttgtgttt gttatacgtg taagecegee caatgtgtea tggeegaact tattatggtt 1381 acatatttat gaaatgggct tcattatcaa ttgatttgag cctacaaaaa tgtagccata 1441 aageceatta agtigtaatt gitaatatti eagteataaa taigattite tatatetaig 30 1501 atttatctct agtgttgatg atgtttgtat gtggaagtca tgttctattt gcttccacgg 1561 tttaaaaacc atcaacttgc taaggtcaaa ttctaatatt actgtgaaaa acattattta 1621 cgtgcgtaat tatatgaatt tatgaatagg ttttaattcc atttttcct aatagtgttt 1681 tatgtcaaa

36

MANCIRRNALFFLTLLFLLSVSNLVOAA

40 RGGCKLKPQQ

CNSKCSFRCSATSHKKPCMFFCLKCCKKCLCVPPGTFGNKQTCPCYNNWKTKEGRPKCP*

45

I. At4g09600 (GASA3)

```
1 taggotggca atttaactot gagacgtott tottgtatag agaataaaac atacgogtgt
 5
            61 aaaagaaaac gcgtgaatcg aatgatgagt gttaacgttc gatcgagatg ccaccaaatc
           121 ttttcattaa aatgaattgt ggaggacata ccacttttaa cgaggtcatt tccactgggt
           181 gacatgtgga ctctactttg ggtggcatgt tcatatcttt ccacatcacc atgtaaacgt
           241 'gaaaacaccc accacactca cttacatctc aaacacatgt cttcattatc gtacgtaget
           301 ccaaaaaaaa aaatgaaaac taggtttagt gattctattt cgcaatgtat aatatacaac
10
           361 ttgtaaaaat aaaatatttg aataagcatt ataaataaac ccaaagaggt gttagattta
           421 tatacttaat tgtagctact aaatagagaa tcagagagaa tagttttata tcttgcacga
           481 aactgcatgc tttttgagac ATGGCAATCT TCCGAAGTAC ACTAGTTTTA CTGCTGATCC
           541 TCTTCTGCCT CACCACTTTT GAGgttcata acttttgtct ttacttctcc atgaatcatt
           601 tgcttcgtct tatccttaat tcatatgtgt ttgatcaatg ataataattc atcattctct
15
           661 tcagcttcat gttcatgctg ctgaagattc acaagtcggt gaaggcgtag tgaaaattgg
           721 tatgtaacgc taacatatat gtaaagtgtt atatctctgt ttatatatga tttttaaacg
           781 gttaaaaact agtcatatgt gtataaatat atcatgtgaa gATTGCGGTG GGAGATGCAA
841 AGGTAGATGC AGCAAATCGT CGAGGCCAAA TCTGTGTTTG AGAGCATGCA ACAGCTGTTG
           901 TTACCGCTGC AACTGTGTGC CACCAGGCAC CGCCGGGAAC CACCACCTTT GTCCTTGCTA
20
           961 CGCCTCCATT ACCACTCGTG GTGGCCGTCT CAAGTGCCCT TAAacatata cacatacaga
          1021 tgtgtgtata tgtcttccgc gagcacacac gtacgtttat gttttaagga caatagtatg
          1081 tatgagcagc tataaacaaa ccagaagtta atggttcatg ttgaactagt ataagttgta
          1141 tgaactgtgc ttcttttgaa caaccacttt tgctgtaagt ttagcaaccc tatttaataa
          1201 attagagatt acaaaaaaa aaatgaaaaa tgtttaaaaa acgtggattt ttaaatttgg
25
          1261 gattaaaaat taattttcat tttggttgat ttgtcaataa attagctaag ttttgtatac
          1321 taggeogttt aagatatget gttaaatttt tgataataga gttgeettag aagtteataa
          1381 ctgtaaatat ctaacttcac ttcaatctca caaacacacg aatcaacttc agcactaaga
          1441 atcgaattga ccagaactga aagaaagtaa aagaaaagct gaatacagag aatttaacga
```

MAIFRSTLVLLLILFCLTTF

30

ELHVHAAEDSQVGEGVVKID

35 CGGRCKGRCSKSSRPNLCLRACNSCCYRCNCVPPGTAGNHHLCPCYASITTRGGRLKCP*

J. At4g09610 (GASA2)

```
1 ttaacagttt aacaccataa tgttaaactc ggtttagcat tttggtgtaa ttctacctct
 5
            61 ttaaccatac atactaaaga cgcagagaag ttcatatggt agttaatcgt aaatagctaa
           121 acttttaatt ggggttaaca tattatttaa cacttaacat ttaactattg atctctcatt
           181 tttttttat taaccaaaat aaattcattt tagaaccaaa cgtttcaaaa actcgtaatg
           241 ttttctcatt aaatcttatc tatagctcac acaaagaaaa actacggaca tgcatgcacc
           301 caattatata catggattat tatttttägt gttataatat gatacaaaat aaaaaacatt
10
           361 tggatagccg ataggcgata gccactataa atataccaaa gaggttggat tatacatata
           421 geographia caaagagagt atcagataga aatagtteta atattttgta caactcacag
           481 aaattgcatg agtttcgaac ATGGCAGTCT TCCGAAGTAC ACTGGTTCTG TTACTAATCA
           541 TCGTCTGTCT CACCACTTAT GAGgtttata atatttttgg tctttatagt tccccaagaa
           601 cacctagcaa tattatactc aattcatgtt tatatgataa tgactgatca ttctcttcag
15
           661 CTTCACGTCC ACGCTGCTGA TGGTGCAAAG GTCGGTGAAG GCGTAGTGAA AATCGgtatg
           721 taaccctaac ttatatataa cacgttggta tataacttaa tatttetgat gggtgcacte
           781 tcttcccaac ttatatatat ctttgttatg gagaatgtct caagctttta atgagatgtt
           841 atatotogga gaaggaaact atgaactaaa agotttggat tootttgcaa caaatataaa
           901 cttttgatgg gtttaaacgg attaaattag ttacatgtgt ttgatgaatg tatgtatgat
20
           961 tgtagATTGT GGTGGGAGAT GCAAAGATAG ATGCAGCAAA TCTTCGAGAA CGAAGCTATG
          1021 CTTGAGAGCG TGCAACAGCT GTTGTTCCCG CTGCAACTGT GTGCCACCTG GTACTTCTGG
          1081 AAACACCCAC CTTTGTCCTT GCTACGCCTC CATTACCACT CACGGTGGCC GCCTCAAGTG
          1141 CCCTTAAaat ttcttctgtg tctgtttctg tttctacttc tatttcgaat atatgtacat
          1201 gtgtgtgtac gtgtgtatgt atacaagtac tgctatgttt tggaggacaa aagtatatgt
25
          1261 atgagaaget ataaactaat tagaagttga tggttatgeg tattateaaa eegtgttaet
          1321 totgaacaac caattteggt ttgttccaag tttggcaacc ctaaaataaa aattcaaaat
          1381 gattggagac tactcgttaa tagacattga aaacgatgaa atctcgttac gtttttatat
          1441 tttttgaact gtaatattat tatgcagaag cggttttgta atgggccgac aaaaaaaaag
          1501 tggttttgta atggatatga ttcggatcta ttctggaaat ggtctcaaaa agtagagttg
30
          1561 agateteaat acgaaaatga accetttegt ttgatttate aaageetttt attttgaaaa
          1621 cgttaaatcc tcactaggat ctctctt
```

35 MAVERSTLVLLIIVCLTTY

ELHVHAADGAKVGEGVVKID

CGGRCKDRCSKSSRTKLCLRACNSCCSRCNCVPPGTSGNTHLCPCYASITTHGGRLKCP**

40

K. At5g15230 (GASA4)

```
1 aaatattcac ootaaaatga atotaaaaat gtacaaaatc acaggaaaat aaaactaagc
 5
            61 agaaatgtcc taagaaaact aaagttttta aaaaataatc ttcaaagaga tactccaact
           121 ggtqttataa gcaaaacttg atttatcaaa aacaggttca tagtatttta tatttagtac
           181 tataagettt eettaaacca tgtgcaaaac catetaccgc agtetaatta ccaatagcaa
           241 gtaataaaat gggactaaca ttggaggcat acgtggaata atataattgg aggaatacag
           301 taataatgat atgtgttgcc acagggaata attgatacga gcaaatgtgt gtatatatag
           361 cttatatgca acateattgg gtcctcaacc aaaaactcct ctctcagtac acttcttttc
10.
           421 atacetcaag agactaaaac tagtttgagg agatttagag gagtgtttgg ttetttggat
481 aacaatatec caaactgaaa ATGGCTAAGT CATATGGAGC TATCTTCCTC TTGACCCTCA
           541 TTGTCCTCTT CATGCTTCAA ACCATGgtaa cacctctatt attitttet tetttcaatg
           601 tttgaaaata ttgaagataa tatatttgat tgttttcctt attgacgaac gatatgagac
15
           661 aaatgtgggt totattattg tacttttagt tggaatatat ttaatttagc ctttttaatg
           721 aaattaattt tacttgtttt teetetetet tittitegtt tittagGTTA TGGCCTCAAG
           781 TGGATCTAAT GTGAAGTGGA GCCAGgtcag ttttattatt gaatcgacta gtaattacct
           841 tttaaactat attttatacc tattgttatc tcgtaactta acgaaaagtg attaattagt
           901 tacctttttt ggttaatttt cagAAACGTT ATGGACCAGG AAGCCTGAAA CGTACCCgta
20
           961 agtttttct tcacagctat tcttaaacaa tttttttta atctcataat cgacgaaaaa
          1021 taaacaatto aagaaatott ttattgtgtt ataataaaaa aaaataagca tttoagttgo
          1081 agaaaataag ttgaaagtga agtgttaagt ggactgtttg gtcagatccg tagactcaaa
          1141 atatattaga tattgacgaa attgcccctt aatatggtca tacagtcaaa gcaacccact
          1201 atcttgagac ccacaaaaca gtaaaaaaaa aagctaatga atttccacta gattctgttg
          1261 tttttattag taataaaaaa tttttgagtg ttaacatttt gatattgttt gtatttgaaa
25
          1321 CAACCAGAAT GCCCATCGGA ATGTGATAGG AGGTGTAAAA AGACACAGTA CCACAAGGCT
          1381 TGCATTACGT TCTGCAACAA ATGCTGCAGG AAGTGTCTCT GTGTGCCTCC GGGTTACTAT
          1441 GGGAACAAC AAGTTTGCTC CTGCTACAAC AACTGGAAAA CTCAAGAGGG TGGACCAAAA
          1501 TGCCCTTGAa aaaateteee ttegtteeet ttttataata aaaattttea actataacta
30
          1561 aattteettt gateaatgtt ttatetaett tatteetaat gttgtaatgt tatgteacte
          1621 cttttcggat tttgttctaa atcctaaaaa aaatgagagt ggccctatga atgatatttt
          1681 tcatgaatac ttgtgtttct aaagatattt tcccattcat ccaccaaaaa aaaagatatt
          1741 ttccatttcg aaaatagtaa tactataaag ggtaaggcaa accaaataat acaatttaaa
          1801 aaatteetge gaaagaagta tgeatatgta gaaaagagtg acattgggte teteggeeca
35
          1861 gtactaaaaa gcccattatt gatttttcca agctttttac aaaatcacgt gttctaacgc
          1921 gattgetttt tgeegeaate ttettttata caagaettgg getttgggea gttggaaata
          1981 aataacgaca acgatatttt acaatcggt
```

${\tt MAKSYGAIFLLTLIVLFMLQTMV}$

MASSGSNVKWSQKRYGPGSLKRTQ

CPSECDRRCKKTQYHKACITFCNKCCRKCLCVPPGYYGNKQVCSCYNNWKTQEGGPKCP**

40

L. At5g14920

	1	ttactcasta	atacaataat	cgaagtgaag	ancetettta	tatuaaarat	ahaagegaca
5				atgctattta			
•				tggggtactc			
				aaagtattcc			
				atttttggga			
				tgaaggttca			
10				tacqcattta			
				atcacaaaag			
				ATGGCTCTCT			
				tcttactttt			
				tgtgcattac			
15				TTGTTTTTGC			
				taattatttc			
				aagttaaatt			
	841	TTCTTTACCA	ACGCCAACAC	TTCCATCGCC	ATCTCCGGCT	ACCAAACCGC	CGTCGCCAGC
	901	TCTCAAACCG	CCGACGCCGT	CGTACAAGCC	ACCCACGCTG	CCAACTACTC	CTATTAAACC
20	961	ACCCACCACA	AAACCTCCGG	TCAAACCTCC	AACTATTCCG	GTTACACCAG	TAAAACCTCC
	1021	GGTTTCAACT	CCTCCGATCA	AACTACCGCC	GGTACAACCA	CCTACGTACA	AACCCCCAAC
	1081	GCCAACAGTT	AAACCACCGT	CCGTCCAACC	ACCTACGTAC	AAACCCCCAA	CTCCAACGGT
	1141	TAAACCACCC	ACTACATCAC	CGGTTAAACC	ACCCACTACG	CCACCAGTTC	AATCACCGCC
	1201	GGTCCAACCA	CCTACGTACA	AACCCCCAAC	GTCACCGGTT	AAACCACCCA	CCACAACTCC
25	1261	ACCGGTTAAA	CCCCCCACCA	CGACGCCACC	GGTCCAACCA	CCTACGTACA	ATCCCCCAAC
	1321	TACACCGGTT	AAACCACCTA	CAGCGCCGCC	TGTCAAACCT	CCAACACCAC	CTCCCGTAAG
				attttctttc			
				taaattttgg			
				AGGAAGAACG			
30				CCAGGCACCT			
				GGTGGAAAAT			
				taaatcaatg			
				ttggatcttt			
~=				taagggtttt			
35				tctaacaggt			
				tatgtaatag			
				tcaaaagatg			
				ttgcaaatct			
40			gcctatttgg	gccgatgtgt	ttgaagatca	tttgggaaag	tcttgcgcaa
4 0	2161	ggag					

MALSLLSVFIFFHVFTNVVFAAS

45 NEESNALVSLPTPTLPSPSPA
TKPPSPALKPPTPSYKPPTLP
TTPIKPPTTKPPVKPPTIPVT
PVKPPVSTPPIKLPPVQPPTY
KPPTPTVKPPSVQPPTYKPPT
50 PTVKPPTTSPVKPPTTPPVQS
PPVQPPTYKPPTSPVKPPTTT
PPVKPPTTTPPVQPPTYNPPT
TPVKPPTAPPVKPPTPPPVRT
RID

 ${\tt CVPLCGTRCGQHSRKNVCMRACVTCCYRCKCVPPGTYGNKEKCGSCYANMKTRGGKSKCP*}$

M. At5g59845

5	1	gacttgagta	tgaatccaat	aacccaaaat	ttatgcagat	tttagaatac	ttcttataaa
Ð	61	tcttaaatga	ataacacaaa	actttaacat	acttttaaca	aatcttgatt	gaataacaac
	121	agattctaca	tgacatttta	aatcactaaa	actcttttga	aatcataaac	caataacaac
	181	cccttagttt	tttactattt	gaattctgac	gtacttttt	attaqttqaa	tttctataaa
	241	tgagaaaaca	ttaattattt	cttaatcttt	gaacttaagc	cccacaaaaa	tcttataaat
	301	tgggacagat	ggactagata	acaagcgttt	cacctactcc	aaaatttccc	tataaqtaac
10	361	tctttttgta	acctcctttt	cttcccaaac	catcactcct	tttgcattgt	gtgaaacctt
	421	cgagttttct	cttcatcttc	tcaaagtaac	aaactttctc	caaacagatt	attattaaaa
	481	caatctcatc	aagaactacg	ATGAAATTCC	CGGCTGTAAA	AGTTCTTATT	ATCTCTCTTC
	541	TCATCACATC	TTCTTTGTTC	ATACTCTCAA	CCGCGGATTC	GT gtaagtat	acacaatoca
	601	ttttcttatt	ttagatactt	ttctcattag	aaatttagct	ttcttaataa	aattotatto
15	661	tgatgatgga	ttaattagCA	CCATGCGGAG	GAAAATGCAA	CGTGAGATGT	TCAAAGGCAG
	721	GAAGACAAGA	TAGGTGTCTC	AAGTATTGTA	ATATATGTTG	CGAGAAGTGT	AACTATTGTG
	781	TTCCTTCAGG	CACTTATGGA	AACAAAGATG	AATGCCCTTG	TTACCGCGAT	ATGAAGAACT
	841	CCAAAGGCAC	GTCCAAATGT	CCTTGAtcat	gttcttaaga	ttatccttat	agacacaata
	901	tcttgaaatg	ttaagattgt	gcttgatgcc	taaaataato	accttoacat	acttctatga
20	961	atgaatatgt	gaaagatttt	gacaataaaa	tgatttgatg	tattaaaata	ttcttagtga
	1021	agttatatat	gtataaatqa	agtatgaaat	atacattota	tottocttta	Catgagaaag
	1081	ataaatctac	aacaatccaa	tgtatgaaaa	ttttactaag	ttaactoatc	agaaacgtta
	1141	attatggttt	agaatcttqt	qqaqaqatqa	ttacttttgt	aagagaaatt	gattotttot
	1201	tgtcaatgag	gataaagtaa	gaagccattt	ctcaacacat	ggacttgata	gcaaactaaa
25	1261	caaggotcaa	gcattqaaat	tgaaacgtct	cqataqataa	gattggctca	agaaaagcaa
	1321	gtgttttttg	ttgtagaaaa	cagaaattga	aattactgtc	tacttt	-555000

30 MKFPAVKVLIISLLITSSLFILSTA

DSSP

35

 ${\tt CGGKCNVRCSKAGRQDRCLKYCNICCEKCNYCVPSGTYGNKDECPCYRDMKNSKGTSKCP*}$

N. At3g10170

genomic structure before splicing and processing 5' - towards 3'predicted orf sequences are underlined

5

CTGTTTTCAGAAAATGGCAACAAAACTTAGCATCATTGTTTTCTCCATTG TTGTGTTACATCTTCTGTCTGCCCATATGCATGTAAGTGTTTCAACA CTCTATTCCTCTATGTTCACATTTATCAACTTTATCTTATACGTCCCTGA ATAAAACACAGCCTATATACTTGGAATCTCCTGCTCGACAACCACAACCA 10 CCACAGTCGCAACCACAACTGCCGCATCACAATAACTCTCAAGTGAGTTT CTCGGTTCATCACTACTCAAAAAAAGAGTTTCATCGAATCTACAAAACCT TTTTAACATCCTTTGCATCTTCTTGTTGATTTTGGCAGTACGGTACTACT CAAGGCAGTCTTCAACCCCAAGGTAAACCCACTGACTAGCCTAGTTTTTA ATTAATGTTTGTGCTGAATGCGAAACTAAATCCGCTATTCCACCTTTATT 15 AGAGTGCGGGCCAAGGTGTGGAGATAGATGCTCGAATACAAGA CCCCCAGGTACTTATGGCAATAAGCAAGTATGTCCTTGCTATAACAACTG

GAAGACCAAGAGCGGTGGACCAAAATGCCCTTAGTTTCTCCTCTTAATTA CTTTAGCATAAACTCCATGTAATTTGTTAATCTACCTATCATAATTTATA 20

TATGTATTGGACTCTTCCATAATCACATCAGTTCTCTGTGATTATGACGT

Amino acid sequence of the predicted pre-pro-peptide the first line represents the signal sequence the second (set of) lines represents the the pro-peptide 25 the last line represents the conserved Cysteine motif.

MATKLSIIVFSIVVLHLLLSAHMH

FLINVCAECETKSAIPPLLE

30

 ${\tt CGPRCGDRCSNTQYKKPCLFFCNKCCNKCLCVPPGTYGNKQVCPCYNNWKTKSGGPKCP*}$

They consist of an N-terminal signal peptide, followed by a variable domain (involved in mobility or cell wall attachment) and a C-terminal domain with 12 conserved cystein residues.

The consensus of this last domain is:

C-C-RC------G--KCP*

- (-) = any amino acid;
- (C) = conserved C-residue
- 10 (/) = either one or the other amino acid at this position;
 - * = stopcodon

Some members of this gene family have been described previously, and represent the GASA family in Arabidopsis 15 thaliana (Plant Mol. Biol. 36 (1998). Similar family members containing the same structural motifs are present in rice (like GASR1) and tomato (Plant Journal 2 (1992) 153-159; Mol. Gen. Genet. 243 (1994) Taylor and Scheuring). In Arabidopsis, the GASA gene family represents 14 different 20 membres, similar as the number for the RKS gene family. Our data on the similar phenotypes for RKS4 and GASA3 (figure 6) and the fact that there are similar numbers of ligands and receptors suggest that there is a single GASA ligand molecule interaction with a single RKS molecule. T-DNA knock out 25 phenotypes observed with several of the other GASA peptide ligand genes also show modifications of organ and plant size like the appearance of extreme dwarf plants resembling brassinosteroid insensitive mutants. Co-localization of RKS genes and GASA ligands on the genome (see figure 4) could 30 provide clues of molecular interactions between GASA molecules and RKS molecules (similar as for S locus proteins and S locus receptor kinases).

Furthermore, in the chapter discussing the effects of roots in RKS transgenic plants, it was shown that overexpression of RKS genes can result in the formation of lateral roots (figure 26). One of the GASA ligands is involved in the formation and/or outgrowth of lateral roots as discussed in Mol. Gen. Genet. 243, 1994, 148-157.

Intracellularly, this signal is transmitted onto membrane (but not necessarily plasma membrane) associated NDR-NHL proteins. At least some of the functions of the syntaxin-like NDR-NHL proteins would thereby result in the regulation of vesicle transport and /or the positioning of new cell wall formation. Neighboring cells are known to influence and determine the developmental state and the differentiation of cells. In transgenic plants with RKS and / or NDR-NHL expression cassettes the positioning of new cell walls is modified, resulting in abnormal neighboring cells, resulting in abnormal development of groups of cells like flower meristem primordia as observed and shown with RKSO, RKS13 and NHL1O.

10

and in rice:
ŗņ
and
arabidopsis
in
genes
complex
ıal
signa
ທ
RK
of RKS
numbers of RK
accessions numbers of RK
m
overview of accessions numbers of RK
iew o

	Gene	Gene code	contig	gene prediction	diction	Oryza sativa	approximate position
ro				in At database	ıtabase	japonica contig	in bp around:
	RKS0	At1g71830	f14o23	ok		OSJNBa0036B21	52.000
	RKS1	At1960800	£8a5	ok		P0038C05	60.000
	RKS2	At5g65240	mqn23	ok		OJ1212 C08	8000
	RKS3	At5g63710	mbk5	ok		see rks2	
10	RKS4	At2g23950	t29e15	wrong, e	wrong, exon missing	P0708B04	35.000
	RKS5		mra19	wrong,	exon missing	0J1077_A12	102.000
	RKS6		wt e 23	ok		see rks2	
	RKS7	At5g16000	ku e 24	ok		P0038C05	60.000
	RKS8		£23m19	ok		OJ1134 B10	90.000 & 1000 2
15	diffe	different genes				I	
	RKS1(RKS10 At4g33430	en d 25	wrong,	exon missing	see rks0	
	RKS11	RKS11 At4g30520	wu d 20	wrong,	exon missing	see rks4	
	RKS12	RKS12 At2g13800	£13j11	wrong,	exon missing	see rks10	
	RKS13	RKS13 At2g13790	£13j11	ok		P0633E08	36.000
20	RKS14	RKS14 At3g25560	mw12	wrong,	exon missing	OSJNBb0015G09	36.000
	ELS1	At5g21090	ch e 52	ok		P0003H10	53.000
	ELS2		allelic variant c	f ELS1 no	possibly allelic variant of ELS1 no genomic sequence identified yet	identified yet	see els1
	ELS3	At3g43740 by c 21	by c 21	ok		P0468B07	52.000

Homology between aa sequences from arabidopsis proteins are compared with the rice databases using: http://mips.gsf.de/proj/thal/db/search/search frame.html protein sequences based on Oryza sativa japonica contig sequences.

30

25

Arabidopsis thaliana ELS1 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase

10 letters.

30

ttactctcaaattccttttcgatttccctctcttaaacctccgaaagctcac **ATG**GCGTCTCGAAACTATCGGTGGGAGCTCTTCGCAGCTTCGTTAACCCTAA CCTTAGCTTTGATTCACCTGGTCGAAGCAAACTCCGAAGGAGATGCTCTCTA 15 CGCTCTTCGCCGGAGTTTGACAGATCCAGACCATGTCCTCCAGAGCTGGGAT CCAACTCTTGTTAATCCTTGTACCTGGTTCCATGTCACCTGTAACCAAGACA ${\tt ACCGCGTCACTCGTGTGGATTTGGGAAATTCAAACCTCTCTGGACATCTTGC}$ GCCTGAGCTTGGGAAGCTTGAACATTTACAGTATCTAGAGCTCTACAAAAAC AACATCCAAGGAACTATACCTTCCGAACTTGGAAATCTGAAGAATCTCATCA GCTTGGATCTGTACAACAACAATCTTACAGGGATAGTTCCCACTTCTTTGGG 20 AAAATTGAAGTCTCTGGTCTTTTTACGGCTTAATGACAACCGATTGACGGTC CAATCCCTAGAGCACTCACGGCAATCCCAAGCCTTTAAAGTTGTGACGTCTC AAGCAATGATTTGTGTGGACAATCCCACAAACGGACCCTTTGCTCACATTCC TTTACAGAACTTTGAGAACAACCCGAGATTGGAGGGACCGGAATTACTCGGT 25 CTTGCAAGCTACGACACTAACTGCACCTGAacaactggcaaaacctgaaaat gaagaattggggggtgaccttgtaagaacacttcaccactttatcaaatatc

Predicted amino acid sequence of the Arabidopsis thaliana ELS1 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt *et al.* (1997).

At the predicted extracellular domain the first domain
represents a signal sequence. The second domain contains a
leucine zipper motif, containing 4 leucine residues, each
separated by seven other amino acids. The third domain
contains conserved cysteine residues, involved in disulphate
bridge formation. The fourth domain contains a leucine rich

repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The last domain might be involved in attachment to other proteins or structures within the cell wall.

5

MASRNYRWELFAASL TLTLALIHLVEANSEG

DALYALRRSLTDP

10 DHVLQSWDPTLVN

PCTWFHVTCNQDNRVTRV

DLGNSNLSGHLA

15 P ELGKLEHLQYLELYKNNIQGTI
PSELGNLKNLISLDLYNNNLTGIV
PTSLGKLKSLVFLRLNDNRLTGPI
PRALTAIPSLKVVDVSSNDLCGTI
PTNGPFAHIPLQNFENNPRLEGPE

20

LLGLASYDTNCT

Arabidopsis thaliana ELS2 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 aaaattactcaaattcctattagattactctcttcgacctccgatagctcac ${\bf ATG} {\tt GCGTCTCGAAACTATCGGTGGGAGCTCTTCGCAGCTTCGTTAATCCTAA}$ CCTTAGCTTTGATTCACCTGGTCGAAGCAAACTCCGAAGGAGATGCTCTTTA CGCTCTTCGCCGGAGTTTAACAGATCCGGACCATGTCCTCCAGAGCTGGGAT CCAACTCTTGTTAATCCTTGTACCTGGTTCCATGTCACCTGTAACCAAGACA ACCGCGTCACTCGTGGGGATTTGGGGAATTCAAACCTCTCTGGACATCTTGC GCCTGAGCTTGGGAAGCTTGAACATTTACAGTATCTAGAGCTCTACAAAAAC AACATCCAAGGAACTATACCTTCCGAACTTGGAAATCTGAAGAATCTCATCA GCTTGGATCTGTACAACAACAATCTTACAGGGATAGTTCCCACTTCTTTGGG AAAATTGAAGTCTCTGGTCTTTTTACGGCTTAATGACAACCGATTGACGGGG 20 ${\tt CAATCCCTAGAGCACTCACTGCCAATCCCAAGCCTTAAAAGTTGTGGATGTC}$ TAAGCAATGATTTGTGTGGAACAATCCCAACAAACGGACCTTTTGCTCACAT TCCTTTACAGAACTTTGAGAACAACCCGAGGTTGGAGGGACCGGAATTACTC ${\tt GGTCTTGCAAGCTACGACACTAACTGCACC\underline{TGA}} {\tt agaaattggcaaaacctga}$ aaatgaagaattgggggggaccttgtaagaacacttcaccactttatcaaat 25 gaatcgaatagtaatatcatctggtctcaattgagaactttgaggtctgtgt atgaaaattaaagattgtactgtaatgttcggttgtgggattctgagaagta

30

Predicted amino acid sequence of the $Arabidopsis\ thaliana\ {\tt ELS2}$ protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino acids. The third domain

contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The last domain might be involved in attachment to other proteins or structures within the cell wall.

MASRNYRWELFAASL ILTLALIHLVEANSEG

10

DALYALRRSLTDP DHVLQSWDPTLVN

PCTWFHVTCNQDNRVTRV

15

DLGNSNLSGHLA

P ELGKLEHLQYLQLYKNNIQGTI
PSELGNLKNLISLDLYNNNLTGIV
PTSLGKLKSLVFLRLNDNRLTGPI

20 PRALTAIPSLKVVDVSSNDLCGTI
PTNGPFAHIPLQNFENNPRLEGPE

LLGLASYDTNCT

Arabidopsis thaliana ELS3 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 15 AACATTTACAATATCTTGAACTCTACAAAAACGAGATTCAAGGAACTATACCTTCTGAGC

 TTGGAAATCTGAAGAGTCTAATCAGTTTGGATCTGTACAACAACAATCTCACCGGGAAAA

 TCCCATCTTCTTTGGGAAAATTGAAGCGGCTTAACGAAAACCGATTGACCGGTCCTATTC

 CTAGAGAACTCACAGTTATTTCAAGCCTTAAAGTTGTTGATGTCTCAGGGAATGATTTGT

 GTGGAACAATTCCAGTAGAAGGACCTTTTGAACACATTCCTATGCAAAACTTTGAGAACA
- 20 ACCTGAGATTGGAGGGACCAGAACTACTAGGTCTTGCGAGCTATGACACCAATTGCACTT

 AAaaagaagttgaagaa

Predicted amino acid sequence of the Arabidopsis thaliana ELS3 protein.

- Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).
 - At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a
- 30 leucine zipper motif, containing 2 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each
- approximately 24 amino acid residues. The last domain might be involved in attachment to other proteins or structures within the cell wall.

51

MVAQNSRRELLAASL ILTLALIRLTEANSEG

DALHALRRSLSDP

 ${f 5}$ DNVVQSWDPTLVN

PCTWFHVTCNQHHQVTRL

DLGNSNLSGHLV

10 P ELGKLEHLQYLELYKNEIQGTI

PSELGNLKSLISLDLYNNNLTGKI

P SSLGKLKRLNENRLTGPI

PRELTVISSLKVVDVSGNDLCGTI

PVEGPFEHIPMQNFENNLRLEGPE

15

LLGLASYDTNCT

WO 2004/007712 PCT/NL2003/000524 52

Arabidopsis thaliana RKSO cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 atttttattttatttttactctttgtttgttttaatgctaatgggtttttaaaagggttatcgaaaaaatgagtgagtttgtgttgaggttgtctctgtaaagtgttaatggtggtgat $\verb|tttcggaagttagggttttctcggatctgaagagatcaaatcaagattcgaaatttacca|\\$ ttgttgttgaaATGGAGTCGAGTTATGTGGTGTTTATCTTACTTTCACTGATCTTACTT CCGAATCATTCACTGTGGCTTGCTTCTGCTAATTTGGAAGGTGATGCTTTGCATACTTTG AGGGTTACTCTAGTTGATCCAAACAATGTCTTGCAGAGCTGGGATCCTACGCTAGTGAAT 15 CCTTGCACATGGTTCCATGTCACTTGCAACAACGAGAACAGTGTCATAAGAGTTGATTTG GGGAATGCAGAGTTATCTGGCCATTTAGTTCCAGAGCTTGGTGCTCAAGAATTTGCAG TATTTGGAGCTTTACAGTAACAACATAACTGGCCCGATTCCTAGTAATCTTGGAAATCTG ACAAACTTAGTGAGTTTGGATCTTTACTTAAACAGCTTCTCCGGTCCTATTCCGGAATCA TTGGGAAAGCTTTCAAAGCTGAGATTTCTCCGGCTTAACAACAACAGTCTCACTGGGTCA 20 ATTCCTATGTCACTGACCAATATTACTACCCTTCAAGTGTTAGATCTATCAAATAACAGA $\tt CTCTCTGGTTCAGTTCCTGACAATGGCTCCTTCTCACTCTTCACACCCATCAGTTTTGCT$ AATAACTTAGACCTATGTGGACCTGTTACAAGTCACCCATGTCCTGGATCTCCCCCGTTT TCTCCTCCACCACCTTTTATTCAACCTCCCCAGTTTCCACCCCGAGTGGGTATGGTATA ACTGGAGCAATAGCTGGTGGAGTTGCTGCAGGTGCTGCTTTGCCCTTTGCTGCTCCTGCA 25 ATAGCCTTTGCTTGGTGGCGACGAAGAAGCCCACTAGATATTTTCTTCGATGTCCCTGCC GAAGAAGATCCAGAAGTTCATCTGGGACAGCTCAAGAGGTTTTCTTTGCGGGAGCTACAA GTGGCGAGTGATGGGTTTAGTAACAAGAACATTTTGGGCAGAGGTGGGTTTGGGAAAGTC 30 ACTCCAGGTGGAGAGCTCCAGTTTCAAACAGAAGTAGAGATGATAAGTATGGCAGTTCAT $\verb|CCGCTTGATTGGCCAACGCGGAAGAGAATCGCGCTAGGCTCAGCTCGAGGTTTGTCTTAC|\\$ CTACATGATCACTGCGATCCGAAGATCATTCACCGTGACGTAAAAGCAGCAAACATCCTC 35 TTAGACGAAGAATTCGAAGCGGTTGTTGGAGATTTCGGGTTGGCAAAGCTTATGGACTAT AAAGACACTCACGTGACAACAGCAGTCCGTGGCACCATCGGTCACATCGCTCCAGAATAT CTCTCAACCGGAAAATCTTCAGAGAAAACCGACGTTTTCGGATACGGAATCATGCTTCTA ATGTTACTTGACTGGGTGAAAGGATTGTTGAAGGAGAAGAAGCTAGAGATGTTAGTGGAT 40 CCAGATCTTCAAACAACTACGAGGAGAGAGAACTGGAACAAGTGATACAAGTGGCGTTG

WO 2004/007712 PCT/NL2003/000524 53

CTATGCACGCAAGGATCACCAATGGAAAGACCAAAGATGTCTGAAGTTGTAAGGATGCTG GAAGGAGATGGGCTTGCGGAGAAATGGGACGAATGGCAAAAAGTTGAGATTTTGAGGGAA

GAGATTGATTTGAGTCCTAATCCTAACTCTGATTGGATTCTTGATTCTACTTACAATTTG

5

15

20

25

Predicted amino acid sequence of the Arabidopsis thaliana RKSO protein.

Different domains are spaced and shown from the N-terminus

towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the Cterminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30

MESSYVVFILLSLILLPNHSL WLASANLEG

DALHTLRVTLVDP

35 NNVLQSWDPTLVN

PCTWFHVTCNNENSVIRV

DLGNAELSGHLV

40 P ELGVLKNLQYLELYSNNITGPI

PSNLGNLTNLVSLDLYLNSFSGPI PESLGKLSKLRFLRLNNNSLTGSI PMSLTNITTLQVLDLSNNRLSGSV PDNGSFSLFTPISFANNLDLCGPV

5

TSHPCPGSPPFSPPPP FIQPPPVSTPSGYGITG

AIAGGVAAGAAL

10 PFAAPAIAFAWW

RRRKPLDIFFDVPAEEDPE VHLGQLKRFSLRELQVAS

15 DGFSNKNILGRGGFGKVYKGRLAD

GTLVAVKRLKEERTPGGELQFQ

TEVEMISMAVHRNLLRLRGFCM

TPTERLLVYPYMANGSVASCLR

ERPPSQPPLDWPTRKRIALGSA

20 RGLSYLHDHCDPKIIHRDVKAA

NILLDEEFEAVVGDFGLAKLMD

 ${\tt YKDTHVTTAVRGTIGHIAPEYL}$

STGKSSEKTDVFGYGIMLLELI

 ${\tt TGQRAFDLARLANDDDVMLLDW}$

VKGLLKEKKLEMLVDPDLQTNY

EERELEQVIQVALLCTQGSPME

RPKMSEVVRMLE

GDGLAEKWDEWQKVEILREEIDLS

30

25

PNPNSDWILDSTYNLHAVELSGPR

WO 2004/007712 PCT/NL2003/000524 55

Arabidopsis thaliana RKS1 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 GGATTTCTGGTTTTTGTATGGTTCTTTGATATCTCTTCTGCTACACTTTCTCCTACTGGT GTTCTTGAGAATTGGGATGTGAATTCAGTTGATCCTTGTAGCTGGAGAATGGTTTCTTGC ACTGATGGCTATGTCTCTCACTGGATCTTCCTAGCCAAAGCTTGTCTGGTACATTGTCT CCTAGAATCGGAAACCTCACCTATTTACAATCAGTGGTGTTGCAAAACAATGCAATCACT 15 GGTCCAATTCCGGAAACGATTGGGAGGTTGGAGAAGCTTCAGTCACTTGATCTTTCGAAC AATTCATTCACCGGGGAGATACCGGCCTCACTTGGAGAACTCAAGAACTTGAATTACTTG CGGTTAAACAATAACAGTCTTATAGGAACTTGCCCTGAGTCTCTATCCAAGATTGAGGGA CTCACTCTAGTCGACATTTCGTATAACAATCTTAGTGGTTCGCTGCCAAAAGTTTCTGCC 20 AGAACTTTCAAGGTAATTGGTAATGCGTTAATCTGTGGCCCAAAAGCTGTTTCAAACTGT TCTGCTGTTCCCGAGCCTCTCACGCTTCCACAAGATGGTCCAGATGAATCAGGAACTCGT ACCAATGGCCATCACGTTGCTCTTGCATTTGCCGCAAGCTTCAGTGCAGCATTTTTTGTT TTCTTTACAAGCGGAATGTTTCTTTGGTGGAGATATCGCCGTAACAAGCAAATATTTTTT GACGTTAATGAACAATATGATCCAGAAGTGAGTTTAGGGCACTTGAAGAGGTATACATTC 25 AAAGAGCTTAGATCTGCCACCAATCATTTCAACTCGAAGAACATTCTCGGAAGAGGCGGA TACGGGATTGTGTACAAAGGACACTTAAACGATGGAACTTTGGTGGCTGTCAAACGTCTC TTGGCTCTTCATCGCAATCTCCTCCGGCTCCGCGGTTTCTGTAGTAGCAACCAGGAGAGA ATTTTAGTCTACCCTTACATGCCAAATGGGAGTGTCGCATCACGCTTAAAAGATAATATC 30 CGTGGAGAGCCAGCATTAGACTGGTCGAGAAGGAAGAAGATAGCGGTTGGGACAGCGAGA GGACTAGTTTACCTACACGAGCAATGTGACCCGAAGATTATACACCGCGATGTGAAAGCA GCTAACATTCTGTTAGATGAGGACTTCGAAGCAGTTGTTGGTGATTTTGGGTTAGCTAAG CTTCTAGACCATAGAGACTCTCATGTCACAACTGCAGTCCGTGGAACTGTTGGCCACATT GCACCTGAGTACTTATCCACGGGTCAGTCCTCAGAGAAGACTGATGTCTTTGGCTTTGGC 35 ATACTTCTCCTTGAGCTCATTACTGGTCAGAAAGCTCTTGATTTTGGCAGATCCGCACAC CAGAAAGGTGTAATGCTTGACTGGGTGAAGAAGCTGCACCAAGAAGGGAAACTAAAGCAG TTAATAGACAAAGATCTAAATGACAAGTTCGATAGAGTAGAACTCGAAGAAATCGTTCAA GTTGCGCTACTCTGCACTCAATTCAATCCATCTCATCGACCGAAAATGTCAGAAGTTATG AAGATGCTTGAAGGTGACGGTTTGGCTGAGAGATGGGAAGCGACGCAGAACGGTACTGGT 40 GAGCATCAGCCACCGCCATTGCCACCGGGGATGGTGAGTTCTTCGCCGCGTGTGAGGTAT

WO 2004/007712 PCT/NL2003/000524 56

TACTCGGATTATATTCAGGAATCGTCTCTTGTAGTAGAAGCCATTGAGCTCTCGGGTCCT

CGA<u>TGA</u>ttatgactcactgtttttaaaaaa

5 Predicted amino acid sequence of the Arabidopsis thaliana RKS1 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

- At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate
- bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-
- glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably
- also containing sequences for protein / protein interactions.

 The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30

MEGVRFVVWRLGFL VFVWFFDISSATLSPTGVNYEV

TALVAVKNELNDP

35 YKVLENWDVNSVD

PCSWRMVSCTDGYVSSL

DLPSQSLSGT

LSPRIGNLTYLQSVLQNNAITGPI

PETIGRLEKLQSLDLSNNSFTGEI PASLGELKNLNYLRLNNNSLIGTC

5 PESLSKIEGLTLVDISYNNLSGSL

PKVSARTFK VIGNALICGPK

AVSNCSAVPEPLTL

PODGPDESGTRING

10

HHVALAFAASFS

AAFFVFFTSGMFLWW

RYRRNKQIFFDVNEQYDPE

15 VSLGHLKRYTFKELRSAT

NHFNSKNILGRGGYGIVYKGHLND

GTLVAVKRLKDCNIAGGEVQFQ

TEVETISLALHRNLLRLRGFCS

20 SNQERILVYPYMPNGSVASRLK

DNIRGEPALDWSRRKKIAVGTA

RGLVYLHEOCDPKIIHRDVKAA

NILLDEDFEAVVGDFGLAKLLD

HRDSHVTTAVRGTVGHIAPEYL

25 STGQSSEKTDVFGFGILLLELI

TGQKALDFGRSAHQKGVMLDW

VKKLHQEGKLKQLIDKDLNDKF

DRVELEEIVQVALLCTQFNPSH

RPKMSEVMKMLE

30

GDGLAERWEATQNGTGEHQPPPLPPGMVSSS

PRVRYYSDYIQESSLVVEAIELSGPR

Arabidopsis thaliana RKS2 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

5 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

Italics indicate the presence of an alternatively spliced gene product.

10

15

20

25

30

35

40

 ${\tt tcaattttggtagctcttagaaaa} \textbf{ATG} \texttt{GCTCTGCTTATTATCACTGCCTTAGTTTTTAGT}$ AGTTTATGGTCATCTGTGTCACCAGATGCTCAAGGGGATGCATTATTTGCGTTGAGGAGC TCGTTACGTGCATCTCCTGAACAGCTTAGTGATTGGAACCAGAATCAAGTCGATCCTTGT ACTTGGTCTCAAGTTATTTGTGATGACAAGAAACATGTTACTTCTGTAACCTTGTCTTAC ATGAACTTCTCCTCGGGAACACTGTCTTCAGGAATAGGAATCTTGACAACTCTCAAGACT CTTACATTGAAGGGAAATGGAATAATGGGTGGAATACCAGAATCCATTGGAAATCTGTCT GGTAATCTCAAGAATCTACAGTTCTTCAGGACCTTGAGTAGGAATAACCTTAATGGTTCT ATCCCGGATTCACTTACAGGTCTATCAAAACTGATAAATATTCTGCTCGACTCAAATAAT CTCAGTGGTGAGATTCCTCAGAGTTTATTCAAAATCCCAAAATACAATTTCACAGCAAAC AACTTGAGCTGTGGTGGCACTTTCCCGCAACCTTGTGTAACCGAGTCCAGTCCTTCAGGT GATTCAAGCAGTAGAAAAACTGGAATCATCGCTGGAGTTGTTAGCGGAATAGCGGTTATT CTACTAGGATTCTTCTTCTTTTTTCTTCTGCAAGGATAAACATAAAGGATATAAACGAGAC GTATTTGTGGATGTTGCAGGAACGAACTTTAAAAAAGGTTTGATTTCAGGTGAAGTGGAC AGAAGGATTGCTTTTGGACAGTTGAGAAGATTTGCATGGAGAGAGCTTCAGTTGGCTACA GATGAGTTCAGTGAAAAGAATGTTCTCGGACAAGGAGGCTTTGGGAAAGTTTACAAAGGA TTGCTTTCGGATGGCACCAAAGTCGCTGTAAAAAGATTGACTGATTTTGAACGTCCAGGA GGAGATGAAGCTTTCCAGAGAGAGTTGAGATGATAAGTGTAGCTGTTCATAGGAATCTG CTTCGCCTTATCGGCTTTTGTACAACACAAACTGAACGACTTTTGGTGTATCCTTTCATG CAGAATCTAAGTGTTGCATATTGCTTAAGAGAGATTAAACCCGGGGATCCAGTTCTGGAT TGGTTCAGGAGAACAGATTGCGTTAGGTGCAGCACGAGGACTCGAATATCTTCATGAA CATTGCAACCCGAAGATCATACACAGAGATGTGAAAGCTGCAAATGTGTTACTAGATGAA GACTTGAAGCAGTGGTTGGTGATTTTGGTTTAGCCAAGTTGGTAGATGTTAGAAGGACT AATGTAACCACTCAGGTCCGAGGAACAATGGGTCATATTGCACCAGAATGTATATCCACA GGGAAATCGTCAGAGAAAACCGATGTTTTCGGGTACGGAATTATGCTTCTGGAGCTTGTA ACTGGACAAAGAGCAATTGATTTCTCGCGGTTAGAGGAAGAAGATGATGTCTTATTGCTA GACCATGTGAAGAACTGGAAAGAGAGAGAGATTAGAAGACATAGTAGATAAGAAGCTT GATGAGGATTATATAAAGGAAGAAGTTGAAATGATGATACAAGTAGCTCTGCTATGCACA CAAGCAGCACCGGAAGAACGACCAGCGATGTCGGAAGTAGTAAGAATGCTAGAAGGAGAA GGGCTTGCAGAGAGTGGGAAGAGTGGCAGAATCTTGAAGTGACGAGACAAGAAGAGTTT

 ${\tt CAGAGGTTGCAGAGGAGATTTGATTGGGGTGAAGATTCCATTAATAATCAAGATGCTATT}\\ {\tt GAATTATCTGGTGGAAGATAG}\\ {\tt aaacaaaaaa}\\$

- 5 Predicted amino acid sequence of the Arabidopsis thaliana RKS2 protein.
 - Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).
- 10 At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate
- bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 3 complete and 2 incomplete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site
- for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably
- also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions. Italics indicate an alternatively spliced gene
- 30 product.

MALLIITALVFSSL WSSVSPDAOG

35 DALFALRSSLR
ASPEQLSDWNQNQVD

PCTWSQVICDDKKHVTSV

ì

TLSYMNFSS GTLSSGI

G ILTTLKTLTLKGNGIMGGI

PESIGNLSSLTSLDLEDNHLTDRI

PSTLGNLKNLQFLTLSRNNLNGSI

PDSLTGLSKLINILLDSNNLSGEI

PQSLFKIPKYN FTANNLSCGG

TFPQPCVTESSPSGDSSSRKTG

10

5

IIAGVVSGIAVIL

LGFFFFFFC

KDKHKGYKRDVFVDVAGTNFKKGLISGE

15 VDRRIAFGQLRRFAWRELQLAT

DEFSEKNVLGQGGFGKVYKGLLSD

GTKVAVKRLTDFERPGGDEAFQ

REVEMISVAVHRNLLRLIGFCT

20 TOTERLLVYPFMQNLSVAYCLR

EIKPGDPVLDWFRRKQIALGAA

RGLEYLHEHCNPKIIHRDVKAA

NVLLDEDFEAVVGDFGLAKLVD

VRRTNVTTQVRGTMGHIAPECI

25 STGKSSEKTDVFGYGIMLLELV

TGQRAIDFSRLEEEDDVLLLDH

VKKLEREKRLEDIVDKKLDEDY

IKEEVEMMIQVALLCTQAAPEE

RPAMSEVVRMLE

30

GEGLAERWEEWQNLEVTRQEEFQ

RLQRRFDWGEDSINNQDAIELSGGR

35

Arabidopsis thaliana RKS3 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

5 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 aacaatcagaaattgatcttacaatgtttcATGGCCTTAGCTTTGTGGGAATCACTTCG TCAACAACTCAACCAGATATCGAAGGAGGAGCTCTGTTGCAGCTCAGAGATTCGCTTAAT GATTCGAGCAATCGTCTAAAATGGACACGCGATTTTGTGAGCCCTTGCTATAGTTGGTCT TATGTTACCTGCAGAGGCCAGAGTGTTGTGGCTCTAAATCTTGCCTCGAGTGGATTCACA GGAACACTCTCCCAGCTATTACAAAACTGAAGTTCTTGGTTACCTTAGAGTTACAGAAC AATAGTTTATCTGGTGCCTTACCAGATTCTCTTGGGAACATGGTTAATCTACAGACTTTA 15 AACCTATCAGTGAATAGTTTCAGCGGATCGATACCAGCGAGCTGGAGTCAGCTCTCGAAT CTAAAGCACTTGGATCTCTCATCCAATAATTTAACAGGAAGCATCCCAACACAATTCTTC TCAATCCCAACATTCGATTTTTCAGGAACTCAGCTTATATGCGGTAAAAGTTTGAATCAG CCTTGTTCTTCAAGTTCTCGTCTTCCAGTCACATCCTCCAAGAAAAAGCTGAGAGACATT 20 ACTTTGACTGCAAGTTGTTGCTTCTATAATCTTATTCCTTGGAGCAATGGTTATGTAT CATCACCATCGCGTCCGCAGAACCAAATACGACATCTTTTTTGATGTAGCTGGGGAAGAT GACAGGAAGATTTCCTTTGGACAACTAAAACGATTCTCTTTACGTGAAATCCAGCTCGCA ACAGATAGTTTCAACGAGAGCAATTTGATAGGACAAGGAGGATTTGGTAAAGTATACAGA GGTTTGCTTCCAGACAAAACAAAAGTTGCAGTGAAACGCCTTGCGGATTACTTCAGTCCT 25 GGAGGAGAAGCTGCTTTCCAAAGAGAGATTCAGCTCATAAGCGTTGCGGTTCATAAAAAT CTCTTACGCCTTATTGGCTTCTGCACAACTTCCTCTGAGAGAATCCTTGTTTATCCATAC GACTGGCCAACAAGGAAGCGTGTAGCTTTTGGTTCAGCTCACGGTTTAGAGTATCTACAC GAACATTGTAACCCGAAGATCATACACCGCGATCTCAAGGCTGCAAACATACTTTTAGAC 30 AACAATTTTGAGCCAGTTCTTGGAGATTTCGGTTTAGCTAAGCTTGTGGACACATCTCTG ACTCATGTCACAACTCAAGTCCGAGGCACAATGGGTCACATTGCGCCAGAGTATCTCTGC ACAGGAAAATCATCTGAAAAAACCGATGTTTTTGGTTACGGTATAACGCTTCTTGAGCTT GTTACTGGTCAGCGCGCAATCGATTTTTCACGCTTGGAAGAAGAGAGAAAATATTCTCTTG CTTGATCATATAAAGAAGTTGCTTAGAGAACAGAGACTTAGAGACATTGTTGATAGCAAT 35 ${\tt TTGACTACATATGACTCCAAAGAAGTTGAAACAATCGTTCAAGTGGCTCTTCTCTGCACA}$ CAAGGCTCACCAGAAGATAGACCAGCGATGTCTGAAGTGGTCAAAATGCTTCAAGGGACT GGTGGTTTGGCTGAGAAATGGACTGAATGGGAACAACTTGAAGAAGTTAGGAACAAAGAA GCATTGTTGCTTCCGACTTTACCGGCTACTTGGGATGAAGAAGAAACCACCGTTGATCAA GAATCTATCCGATTATCGACAGCAAGA<u>TGA</u>agaagaaacagagagagaaagatatctatg 40 aaaa

Predicted amino acid sequence of the Arabidopsis thaliana RKS3 protein.

5 Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 4 complete repeats of each

approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular

domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth

domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

MALAFVGITSSTTOPDIEG

30

25

10

15

GALLQLRDSLNDSSNRL KWTRDFVS

PCYSWSYVTCRGQSVVAL

35

NLASSGFTGTLS

P AITKLKFLVTLELQNNSLSGAL PDSLGNMVNLQTLNLSVNSFSGSI

PASWSQLSNLKHLDLSSNNLTGSI PTQFFSIPTFEFSGTQLICGKS

5 LNQPCSSSRLPVTSSKKKLRD

ITLTASCVASIIL FLGAMVMYHHH

10

RVRTKYDIFFDVAGEDDR KISFGQLKRFSLREIQLAT

DSFNESNLIGQGGFGKVYRGLLPD

15 KTKVAVKRLADYFSPGGEAAFQ
REIQLISVAVHKNLLRLIGFCT
TSSERILVYPYMENLSVAYRLR
DLKAGEEGLDWPTRKRVAFGSA

HGLEYLHEHCNPKIIHRDLKAA

20 NILLDNNFEPVLGDFGLAKLVD

TSLTHVTTQVRGTMGHIAPEYL

CTGKSSEKTDVFGYGITLLELV

TGQRAIDFSRLEEEENILLLD

HIKKLLREQRLRDIVDSNLTTY

25 DSKEVETIVQVALLCTQGSPED

RPAMSEVVKMLQ

GTGGLAEKWTEWEQLEEVRNKEALLL

30 PTLPATWDEEETTVDQESIRLSTAR

Arabidopsis thaliana RKS4 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 $\verb|tcttccttctggtaatctaatctaaagcttttc| \textbf{ATG} \texttt{GTGATGAAGATATTC}|$ CCTGAAGTGGAGGCGTTGATAAACATAAAGAACGAGTTACATGATCCACATGGTGTTTTC AAAAACTGGGATGAGTTTTCTGTTGATCCTTGTAGCTGGACTATGATCTCTTGTTCTTCA GACAACCTCGTAATTGGCTTAGGAGCTCCAAGTCAGTCTCTTTCAGGAACTTTATCTGGG 15 TCTATTGGAAATCTCACTAATCTTCGACAAGTGTCATTACAGAACAATAACATCTCCGGT AAAATCCCACCGGAGATTTGTTCTCTTCCCAAATTACAGACTCTGGATTTATCCAATAAC CGGTTCTCCGGTGAAATCCCCGGTTCTGTTAACCAGCTGAGTAATCTCCAATATCTGTTG AACAACACTCATTATCTGGGCCCTTTCCTGCTTCTCTGTCTCAAATCCCTCACCTCTCT TTCTTAGACTTGTCTTATAACAATCTCAGAGGTCCTGTTCCTAAATTTCCTGCAAGGACA 20 TTCAATGTTGCTGGGAACCCTTTGATTTGTAAAAACAGCCTACCGGAGATTTGTTCAGGA TCAATCAGTGCAAGCCCTCTTTCTGTCTCTTTACGTTCTTCATCAGGACGTAGAACCAAC GGGTTCATTTGGTATCGAAAGAACAAAGACGGTTAACGATGCTTCGCATTAACAAGCAA GAGGAAGGGTTACTTGGGTTGGGAAATCTAAGAAGCTTCACATTCAGGGAACTTCATGTA 25 GCTACGGATGGTTTTAGTTCCAAGAGTATTCTTGGTGCTGGTGGGTTTGGTAATGTCTAC AGAGGAAAATTCGGGGATGGGACAGTGGTTGCAGTGAAACGATTGAAAGATGTGAATGGA ACCTCCGGGAACTCACAGTTTCGTACTGAGCTTGAGATGATCAGCTTAGCTGTTCATAGG AATTTGCTTCGGTTAATCGGTTATTGTGCGAGTTCTAGCGAAAGACTTCTTGTTTACCCT TACATGTCCAATGGCAGCGTCGCCTCTAGGCTCAAAGCTAAGCCAGCGTTGGACTGGAAC 30 ACAAGGAAGAAGATAGCGATTGGAGCTGCAAGAGGGTTGTTTTATCTACACGAGCAATGC GATCCCAAGATTATTCACCGAGATGTCAAGGCAGCAAACATTCTCCTAGATGAGTATTTT GAAGCAGTTGTTGGGGATTTTGGACTAGCAAAGCTACTCAACCACGAGGATTCACATGTC ACAACCGCGGTTAGAGGAACTGTTGGTCACATTGCACCTGAGTATCTCTCCACCGGTCAG TCATCTGAGAAAACCGATGTCTTTGGGTTCGGTATACTTTTGCTAGAGCTCATCACAGGA ATGAGAGCTCTCGAGTTTGGCAAGTCTGTTAGCCAGAAAGGAGCTATGCTAGAATGGGTG AGGAAGCTACACAAGGAAATGAAAGTAGAGGAGCTAGTAGACCGAGAACTGGGGACAACC TACGATAGAATAGAAGTTGGAGAGATGCTACAAGTGGCACTGCTCTGCACTCAGTTTCTT CCAGCTCACAGACCCAAAATGTCTGAAGTAGTTCAGATGCTTGAAGGAGATGGATTAGCT GAGAGATGGGCTGCTTCACATGACCATTCACATTTCTACCATGCCAACATGTCTTACAGG ACTATTACCTCTACTGATGGCAACAACCAAACCAAACATCTGTTTGGCTCCTCAGGATTT 40

 ${\tt GAAGATGAAGATGATAATCAAGCGTTAGATTCATTCGCCATGGAACTATCTGGTCCAAGG} \\ {\tt TAG} {\tt taaatcttggacacagaaagaacagatataatatccccatgacttcaatttttgtt}$

- 5 Predicted amino acid sequence of the Arabidopsis thaliana RKS4 protein.
 - Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).
- 10 At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 2 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate
- bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-
- glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably
- also containing sequences for protein / protein interactions.

 The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30

MVVMKLITMKIFSVLLLL CFFVTCSLSSEPRNPEV

EALINIKNELHDP

35 HGVFKNWDEFSVD

PCSWTMISCSSDNLVIGL

GAPSQSLSGTLS

66

G SIGNLTNLRQVSLQNNNISGKI PPEICSLPKLQTLDLSNNRFSGEI PGSVNQLSNLQYLRLNNNSLSGPF PASLSQIPHLSFLDLSYNNLRGPV

PKFPARTFNVAGNPLICKNS

LPEICSGSISASPL SVSLRSSSGRRTN

10

15

5

ILAVALGVSLGFAVSVIL SLGFIWY

RKKQRRLTMLRINKQEE GLLGLGNLRSFTFRELHVAT

DGFSSKSILGAGGFGNVYRGKFGD
GTVVAVKRLKDVNGTSGNSQFR
TELEMISLAVHRNLLRLIGYCA

20 SSSERLLVYPYMSNGSVASRLK
AKPALDWNTRKKIAIGAA
RGLFYLHEQCDPKIIHRDVKAA
NILLDEYFEAVVGDFGLAKLLN
HEDSHVTTAVRGTVGHIAPEYL

25 STGQSSEKTDVFGFGILLLELI
TGMRALEFGKSVSQKGAMLEW
VRKLHKEMKVEELVDRELGTTY
DRIEVGEMLQVALLCTQFLPAH

30

GDGLAERWAASHDHSHFYHANM SYRTITSTDGNNQTKHLFG

RPKMSEVVQMLE

SSGFEDEDDNQALDSFAMELSGPR

35

Arabidopsis thaliana RKS5 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 $\verb|ctagaga| attentiate ttttctacg \verb|ATG| GAGATTTCTTTGATGAAGTTTCTTTTA$ GGAATCTGGGTTTATTATTACTCTGTTCTTGACTCTGTTTCTGCCATGGATAGTCTTTTA TCTCCCAAGGTGGCTGCGTTAATGTCAGTGAAGAACAAGATGAAAGATGAGAAAGAGGTT TCTGAAGGTTTTGTGGTTTCTCTAGAGATGGCTAGTAAAGGATTATCAGGGATACTATCT 15 ACTAGTATTGGGGAATTAACTCATCTTCATACTTTGTTACTTCAGAATAATCAGTTAACT GGTCCGATTCCTTCTGAGTTAGGCCAACTCTCTGAGCTTGAAACGCTTGATTTATCGGGG AATCGGTTTAGTGGTGAAATCCCAGCTTCTTTAGGGTTCTTAACTCACTTAAACTACTTG CGGCTTAGCAGGAATCTTTTATCTGGGCAAGTCCCTCACCTCGTCGCTGGCCTCTCAGGT CTTTCTTCTTGGATCTATCTTTCAACAATCTAAGCGGACCAACTCCGAATATATCAGCA 20 AAAGATTACAGGAAATGCATTTCTTTGTGGTCCAGCTTCCCAAGAGCTTTGCTCAGATGC TACACCTGTGAGAAATGCTGCAATCGATCTGCAGCGACGGGTTTGTCTGAAAAGGACAAT AGCAAACATCACAGCTTAGTGCTCTTTTTGCATTTGGCATTGTTGTTGCCTTTATCATC TCCCTAATGTTTCTCTTCTTCTGGGTGCTTTGGCATCGATCACGTCTCTCAAGATCACAC GTGCAGCAAGACTACGAATTTGAAATCGGCCATCTGAAAAGGTTCAGTTTTCGCGAAATA 25 CAAACCGCAACAAGCAATTTTAGTCCAAAGAACATTTTGGGACAAGGAGGGTTTGGGATG GTTTATAAAGGGTATCTCCCAAATGGAACTGTGGTGGCAGTTAAAAGATTGAAAGATCCG ATTTATACAGGAGAAGTTCAGTTTCAAACCGAAGTAGAGATGATTGGCTTAGCTGTTCAC CGTAACCTTTTACGCCTCTTTGGATTCTGTATGACCCCGGAAGAGAGAATGCTTGTGTAT CCGTACATGCCAAATGGAAGCGTAGCTGATCGTCTGAGAGATTGGAATCGGAGGATAAGC 30 ATTGCACTCGGCGCAGCTCGAGGACTTGTTTACTTGCACGAGCAATGCAATCCAAAGATT ATTCACAGAGACGTCAAAGCTGCAAATATTCTACTTGATGAGGAGCTTTGAAGCAATAGTT GGCGATTTTGGTCTAGCAAAGCTTTTAGACCAGAGAGTTCACATGTCACTACCGCAGTC CGAGGAACCATTGGACACATCGCTCCCGAGTACCTTTCCACTGGACAGTCCTCAGAGAAA ACCGATGTTTTCGGATTCGGAGTACTAATCCTTGAACTCATAACAGGTCATAAGATGATT 35 GATCAAGGCAATGGTCAAGTTCGAAAAGGAATGATATTGAGCTGGGTAAGGACATTGAAA GCAGAGAGAGATTTGCAGAGATGGTGGACAGAGATTTGAAGGGAGAGTTTGATGATTTG GTGTTGGAGGAAGTAGTGGAATTGGCTTTGCTTTGTACACAGCCACATCCGAATCTAAGA CCGAGGATGTCTCAAGTGTTGAAGGTACTAGAAGGTTTAGTGGAACAGTGTGAAGGAGGG TATGAAGCTAGAGCTCCAAGTGTCTCTAGGAACTACAGTAATGGTCATGAAGAGCAGTCC 40 TTTATTATTGAAGCCATTGAGCTCTCTGGACCACGATGAtagacttcatagtgtcttaac

tagtcttcttgattttgttgtcattgtcatggc

Predicted amino acid sequence of the Arabidopsis thaliana RKS5 protein.

5 Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains no leucine zipper motif, in contrast to the other RKS proteins. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues.

15 The fifth domain contains many serine residues, and is likely to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein /

MEISLMKFLFLGIWVYYYS VLDSVSAMDSLLSPKV

protein interactions.

30

25

10

AALMSVKNKMKDE KEVLSGWDINSVD

PCTWNMVGCSSEGFVVS

35

LEMASKGLSGILS

T SIGELTHLHTLLLQNNQLTGPI PSELGQLSELETLDLSGNRFSGEI PASLGFLTHLNYLRLSRNLLSGQV PHLVAGLSGLSFLDLSFNNLSGPT
PNISAK DYRKCISLWSSFPR

ALLRCYTCEKCCNR

5 SAATGLSEKDNSK

HHSLVLSFAFGIVV AFIISLMFLFFWVLWH

10 RSRLSRSHVQQDYEF EIGHLKRFSFREIQTAT

SNFSPKNILGQGGFGMVYKGYLPN
GTVVAVKRLKDPIYTGEVQFQ

15 TEVEMIGLAVHRNLLRLFGFCM
TPEERMLVYPYMPNGSVADRLR
DWNRRISIALGAA
RGLVYLHEQCNPKIIHRDVKAA
NILLDESFEAIVGDFGLAKLLD

NILLDESFEAIVGDFGLAKLLD

20 QRDSHVTTAVRGTIGHIAPEYL

STGQSSEKTDVFGFGVLILELI

TGHKMIDQGNGQVRKGMILSW

VRTLKAEKRFAEMVDRDLKGEF

DDLVLEEVVELALLCTQPHPNL

25 RPRMSQVLKV

LEGLVEQCEGGYEARA

PASVSRNYSNGHEEQSFIIEAIELSGPR

Arabidopsis thaliana RKS6 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

attgtttccttcttttgggattttctccttggatggaaccagctcaattaatgagatgag 10 ATGAGAATGTTCAGCTTGCAGAAGATGGCTATGGCTTTTACTCTCTTGTTTTTTTGCCTGT TTATGCTCATTTGTGTCTCCAGATGCTCAAGGGGATGCACTGTTTGCGTTGAGGATCTCC TTACGTGCATTACCGAATCAGCTAAGTGACTGGAATCAGAACCAAGTTAATCCTTGCACT TGGTCCCAAGTTATTTGTGATGACAAAAACTTTGTCACTTCTCTTACATTGTCAGATATG AACTTCTCGGGAACCTTGTCTTCAAGAGTAGGAATCCTAGAAAATCTCAAGACTCTTACT TTAAAGGGAAATGGAATTACGGGTGAAATACCAGAAGACTTTGGAAATCTGACTAGCTTG 15 ACTAGTTTGGATTTGGAGGACAATCAGCTAACTGGTCGTATACCATCCACTATCGGTAAT CTCAAGAAACTTCAGTTCTTGACCTTGAGTAGGAACAAACTTAATGGGACTATTCCGGAG TCACTCACTGGTCTTCCAAACCTGTTAAACCTGCTGCTTGATTCCAATAGTCTCAGTGGT CAGATTCCTCAAAGTCTGTTTGAGATCCCAAAATATAATTTCACGTCAAACAACTTGAAT TGTGGCGGTCGTCAACCTCACCCTTGTGTATCCGCGGTTGCCCATTCAGGTGATTCAAGC 20 AAGCCTAAAACTGGCATTATTGCTGGAGTTGTTGCTGGAGTTACAGTTGTTCTCTTTTGGA ATCTTGTTGTTCTGTTCTGCAAGGATAGGCATAAAGGATATAGACGTGATGTTTTGTG GATGTTGCAGGTGAAGTGGACAGGAGAATTGCATTTGGACAGTTGAAAAGGTTTGCATGG AGAGAGCTCCAGTTAGCGACAGATAACTTCAGCGAAAAGAATGTACTTGGTCAAGGAGGC TTTGGGAAAGTTTACAAAGGAGTGCTTCCGGATACACCCAAAGTTGCTGTGAAGAGATTG ACGGATTTCGAAAGTCCTGGTGGAGATGCTGCTTTCCAAAGGGAAGTAGAGATGATAAGT GTAGCTGTTCATAGGAATCTACTCCGTCTTATCGGGTTCTGCACCACACAAACAGAACGC GCAGGCGACCCGGTTCTAGATTGGGAGACGAGGAAACGGATTGCCTTAGGAGCAGCGCGT GGTTTTGAGTATCTTCATGAACATTGCAATCCGAAGATCATACATCGTGATGTGAAAGCA 30 CTAGTAGATGTTAGAAGGACTAATGTGACTACTCAAGTTCGAGGAACAATGGGTCACATT GCACCAGAATATTTATCAACAGGGAAATCATCAGAGAGAACCGATGTTTTCGGGTATGGA ATTATGCTTCTTGAGCTTGTTACAGGACAACGCGCAATAGACTTTTCACGTTTGGAGGAA 35 GTGAGGATGTTAGAAGGAGAAGGGCTTGCGGAGAGATGGGAAGAGTGGCAAAACGTGGAA GTCACGAGACGTCATGAGTTTGAACGGTTGCAGAGGAGATTTGATTGGGGTGAAGATTCT 40 ATGCATAACCAAGATGCCATTGAATTATCTGGTGGAAGATGAccaaaaacatcaaacctt

PCT/NL2003/000524

Predicted amino acid sequence of the Arabidopsis thaliana RKS6 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

At the predicted extracellular domain the first domain

represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-

glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single

25 leucine rich repeat, probably involved in protein / protein interactions.

MRMFSL

QKMAMAFTLLFFACLCSFVSPDAQG

30

10

15

DALFALRISLRALP NQLSDWNQNQVN

PCTWSQVICDDKNFVTSL

35

TLSDMNFSGTLSSRV GILENLKTLTLKGNGITGEI PEDFGNLTSLTSLDLEDNOLTGRI PSTIGNLKKLQFLTLSRNKLNGTI PESLTGLPNLLNLLLDSNSLSGQI PQSLFEIPKYNFTSNNLNCGG

5 ROPHPCVSAVAHSGDSSKPKTG

IIAGVVAGVTVVL FGILLFLFC

10 KDRHKGYRRDVFVDVAGE VDRRIAFGQLKRFAWRELQLAT

DNFSEKNVLGQGGFGKVYKGVLPD
TPKVAVKRLTDFESPGGDAAFQ

15 REVEMISVAVHRNLLRLIGFCT
TQTERLLVYPFMQNLSLAHRLR
EIKAGDPVLDWETRKRIALGAA
RGFEYLHEHCNPKIIHRDVKAA
NVLLDEDFEAVVGDFGLAKLVD

20 VRRTNVTTQVRGTMGHIAPEYL
STGKSSERTDVFGYGIMLLELV
TGQRAIDFSRLEEEDDVLLLDH
VKKLEREKRLGAIVDKNLDGEY

GEGLAERWEEWQNVEVTRRHEFE

IKEEVEMMIQVALLCTQGSPED

RPVMSEVVRMLE

RLQRRFDWGEDSMHNQDAIELSGGR

25

Arabidopsis thaliana RKS7 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 TGATGATAACAAGATCTTTCTTTTGCTTCTTGGGATTTTTATGCCTTCTCTGCTCTTCTG TTCACGGATTGCTTTCTCCTAAAGGTGTTAACTTTGAAGTGCAAGCTTTGATGGACATAA AAGCTTCATTACATGATCCTCATGGTGTTCTTGATAACTGGGATAGAGATGCTGTTGATC $\tt CTTGTAGTTGGACAATGGTCACTTGTTCTTCTGAAAACTTTGTCATTGGCTTAGGCACAC$ 15 CAAGTCAGAATTTATCTGGTACACTATCTCCAAGCATTACCAACTTAACAAATCTTCGGA TTGTGCTGTTGCAGAACAACAACATAAAAGGAAAAATTCCTGCTGAGATTGGTCGGCTTA CGAGGCTTGAGACTCTTGATCTTTCTGATAATTTCTTCCACGGTGAAATTCCTTTTTCAG TTCCTCTGTCACTATCTAATATGACTCAACTTGCCTTTCTTGATTTATCATACAACAATC 20 TTAGTGGTCCTGTTCCAAGATTTGCTGCAAAGACGTTTAGCATCGTTGGGAACCCGCTGA TATGTCCAACGGGTACCGAACCAGACTGCAATGGAACAACATTGATACCTATGTCTATGA ACTTGAATCAAACTGGAGTTCCTTTATACGCCGGTGGATCGAGGAATCACAAAATGGCAA TCTGGTGGAGACAAAGACATAACCAAAACACATTCTTTGATGTTAAAGATGGGAATCATC ATGAGGAAGTTTCACTTGGAAACCTGAGGAGATTTGGTTTCAGGGAGCTTCAGATTGCGA 25 CCAATAACTTCAGCAGTAAGAACTTATTGGGGAAAGGTGGCTATGGAAATGTATACAAAG GAATACTTGGAGATAGTACAGTGGTTGCAGTGAAAAGGCTTAAAGATGGAGGAGCATTGG GAGGAGAGATTCAGTTTCAGACAGAAGTTGAAATGATCAGTTTAGCTGTTCATCGAAATC TCTTAAGACTCTACGGTTTCTGCATCACACAAACTGAGAAGCTTCTAGTTTATCCTTATA 30 TGTCTAATGGAAGCGTTGCATCTCGAATGAAAGCAAAACCTGTTCTTGACTGGAGCATAA GGAAGAGGATAGCCATAGGAGCTGCAAGAGGGCTTGTGTATCTCCATGAGCAATGTGATC ${\tt CGAAGATTATCCACCGCGATGTCAAAGCAGCGAATATACTTCTTGATGACTACTGTGAAG}$ $\tt CTGTGGTTGGCGATTTTGGTTTAGCTAAACTCTTGGATCATCAAGATTCTCATGTGACAA$ CCGCGGTTAGAGGCACGGTGGGTCACATTGCTCCAGAGTATCTCTCAACTGGTCAATCCT 35 $\tt CTGAGAAAACAGATGTTTTTGGCTTCGGGATTCTTCTTCTTGAGCTTGTAACCGGACAAA$ GAGCTTTTGAGTTTGGTAAAGCGGCTAACCAGAAAGGTGTGATGCTTGATTGGGTTAAAA AGATTCATCAAGAGAAGAAACTTGAGCTACTTGTGGATAAAGAGTTGTTGAAGAAGAAGA GCTACGATGAGATTGAGTTAGACGAAATGGTAAGAGTAGCTTTGTTGTGCACACAGTACC TGCCAGGACATAGACCAAAAATGTCTGAAGTTGTTCGAATGCTGGAAGGAGATGGACTTG CAGAGAAATGGGAAGCTTCTCAAAGATCAGACAGTGTTTCAAAATGTAGCAACAGGATAA 40

 ${\tt ATGAATTGATGTCATCTTCAGACAGATACTCTGATCTTACCGATGACTCTAGTTTACTTG}$ TGCAAGCAATGGAGCTCTCTGGTCCTAGATGAaatctatacatgaatctgaagaagaaga agaacatgcatctgtttcttgaatcaagagggattcttgtttttttgtataatagagagg ttttttggagggaaatgttgtgtctctgtaactgtataggcttgttgtgtaagaagttattactgcacttagggttaattcaaagttctttacataaaaaatgattagttgcgttgaata

10 Predicted amino acid sequence of the Arabidopsis thaliana RKS7 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as

described in Schmidt et al (1997). At the predicted extracellular domain the first domain 15 represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for Oglycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine

protein kinase domain (Schmidt et al. 1997) and is probably 30 also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

35

20

25

MESTIVMMMMITRSFF CFLGFLCLLCSSVHGLLSPKGVNFEV QALMDIKASLHDP HGVLDNWDRDAVD

PCSWTMVTCSSENFVIG

5

10

PRFAA

LGTPSQNLSGTL

KTFSIVGNPLICPT

SPSITNLTNLRIVLLQNNNIKGKI PAEIGRLTRLETLDLSDNFFHGEI PFSVGYLQSLQYLRLNNNSLSGVF PLSLSNMTQLAFLDLSYNNLSGPV

GTEPDCNGTTLIPMSMNL NQTGVPLYAGGSRNHKMA

15

IAVGSSVGTVSLIFIAVGLFLWW

RQRHNQNTFFDVKDGNHHE EVSLGNLRRFGFRELQIAT

20

NNFSSKNLLGKGGYGNVYKGILGD STVVAVKRLKDGGALGGEIQFQ TEVEMISLAVHRNLLRLYGFCI TQTEKLLVYPYMSNGSVA

25 SRMKAKPVLDWSIRKRIAIGAA
RGLVYLHEQCDPKIIHRDVKAA
NILLDDYCEAVVGDFGLAKLLD
HQDSHVTTAVRGTVGHIAPEYL
STGQSSEKTDVFGFGILLLELV
30 TGQRAFEFGKAANQKGVMLDW

VKKIHQEKKLELLVDKELLKKKSY DEIELDEMVRVALLCTQYLPGH RPKMSEVVRMLE

35 GDGLAEKWEASQRSDS VSKCSNRINELMSSS

DRYSDLTDDSSLLVQAMELSGPR

Arabidopsis thaliana RKS8 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 **ATG**GGGAGAAAAAGTTTGAAGCTTTTGGTTTTGTCTGCTTAATCTCACTGCTTCTTCTG TTTAATTCGTTATGGCTTGCCTCTTCTAACATGGAAGGTGATGCACTGCACAGTTTGAGA GCTAATCTAGTTGATCCAAATAATGTCTTGCAAAGCTGGGATCCTACGCTTGTTAATCCG TGTACTTGGTTTCACGTAACGTGTAACAACGAGAACAGTGTTATAAGAGTCGATCTTGGG 15 AATGCAGACTTGTCTGGTCAGTTGGTTCCTCAGCTAGGTCAGCTCAAGAACTTGCAGTAC TTGGAGCTTTATAGTAATAACATAACCGGGCCGGTTCCAAGCGATCTTGGGAATCTGACA AACTTAGTGAGCTTGGATCTTTACTTGAACAGCTTCACTGGTCCAATTCCAGATTCTCTA GGAAAGCTATTCAAGCTTCGCTTTCTTCGGCTCAACAATAACAGTCTCACCGGACCAATT CCCATGTCATTGACTAATATCATGACCCTTCAAGTTTTTGGATCTGTCGAACAACCGATTA 20 ${\tt TCCGGATCTGTTCCTGATAATGGTTCCTTCTCGCTCTTCACTCCCATCAGTTTTGCTAAC}$ AACTTGGATCTATGCGGCCCAGTTACTAGCCGTCCTTGTCCTGGATCTCCCCCCGTTTTCT CCTCCACCACCTTTTATACCACCTCCCATAGTTCCTACACCAGGTGGGTATAGTGCTACT GGAGCCATTGCGGGAGGAGTTGCTGCTGGTGCTGCTTTACTATTTGCTGCCCCTGCTTTA GCTTTTGCTTGGTGGCGTAGAAGAAACCTCAAGAATTCTTCTTTGATGTTCCTGCCGAA 25 GAGGACCCTGAGGTTCACTTGGGGCAGCTTAAGCGGTTCTCTACGGGAACTTCAAGTA GCAACTGATAGCTTCAGCAACAAGAACATTTTGGGCCGAGGTGGGTTCGGAAAAGTCTAC ${\tt AAAGGCCGTCTTGCTGATGGAACACTTGTTGCAGTCAAACGGCTTAAAGAAGAGCGAACC}$ ${\tt CCAGGTGGCGAGCTCCAGTTTCAGACAGAAGTGGAGATGATAAGCATGGCCGTTCACAGA}$ AATCTCCTCAGGCTACGCGGTTTCTGTATGACCCCTACCGAGAGATTGCTTGTTTATCCT 30 TACATGGCTAATGGAAGTGTCGCTTCCTGTTTGAGAGAACGTCCACCATCACAGTTGCCT $\tt CTAGCCTGGTCAATAAGACAGCAAATCGCGCTAGGATCAGCGAGGGGTTTGTCTTATCTT$ CATGATCATTGCGACCCCAAAATTATTCACCGTGATGTGAAAGCTGCTAATATTCTGTTG GACGAGGAATTTGAGGCGGTGGTAGGTGATTTCGGGTTAGCTAGACTTATGGACTATAAA GATACTCATGTCACAACGGCTGTGCGTGGGACTATTGGACACATTGCTCCTGAGTATCTC TCAACTGGAAAATCTTCAGAGAAAACTGATGTTTTTGGCTACGGGATCATGCTTTTGGAA 35 CTGATTACAGGTCAGAGAGCTTTTGATCTTGCAAGACTGGCGAATGACGATGACGTTATG $\tt CTCCTAGATTGGGTGAAAGGGCTTTTGAAGGAGAAGAAGCTGGAGATGCTTGTGGATCCT$ GACCTGCAAAGCAATTACACAGAAGCAGAAGTAGAACAGCTCATACAAGTGGCTCTTCTC TGCACACAGAGCTCACCTATGGAACGACCTAAGATGTCTGAGGTTGTTCGAATGCTTGAA 40 GGTGACGGTTTAGCGGAGAAATGGGACGAGTGGCAGAAGTGGAAGTTCTCAGGCAAGAA

-77

 $\label{eq:GTGGAGCTCTCTCACCCCACCTCTGACTGGATCCTTGATTCGACTGATAATCTTCAT} GCTATGGAGTTGTCTGGTCCAAGA<u>TAA</u>acgacattgtaatttgcctaacagaaaagagaa agaacagagaaatattaagagaatcacttctctgtattctt$

5

15

20

25

Predicted amino acid sequence of the Arabidopsis thaliana RKS8 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the Cterminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30 MGRKKFEAFGFVCLISLLLLFNSL WLASSNMEG

> DALHSLRANLVDP NNVLQSWDPTLVN

35

40

PCTWFHVTCNNENSVIRV

DLGNADLSGQLV

P QLGQLKNLQYLELYSNNITGPV PSDLGNLTNLVSLDLYLNSFTGPI PDSLGKLFKLRFLRLNNNSLTGPI PMSLTNIMTLQVLDLSNNRLSGSV PDNGSFSLFTPISFANNLDLCGPV

5 TSRPCPGSPPFSPPPP FIPPPIVPTPGGYSATG

> AIAGGVAAGAAL LFAAPALAFAWW

10

25

RRRKPQEFFFDVPAEEDPE VHLGQLKRFSLRELQVAT

DSFSNKNILGRGGFGKVYKGRLAD

15 GTLVAVKRLKEERTPGGELQFQ
TEVEMISMAVHRNLLRLRGFCM
TPTERLLVYPYMANGSVASCLR
ERPPSQLPLAWSIRQQIALGSA
RGLSYLHDHCDPKIIHRDVKAA
20 NILLDEEFEAVVGDFGLARLMD
YKDTHVTTAVRGTIGHIAPEYL
STGKSSEKTDVFGYGIMLLELI
TGQRAFDLARLANDDDVMLLDW
VKGLLKEKKLEMLVDPDLQSNY

GDGLAEKWDEWQKVEVLRQEVELS

TEAEVEQLIQVALLCTQSSPME

RPKMSEVVRMLE

30 SHPTSDWILDSTDNLHAMELSGPR

Arabidopsis thaliana rks10 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- The first stopcodon has been underlined.

 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 taatctcttgaggataaaATGGAACGAAGATTAATGATCCCTTGCTTCTTTTGGTTGATT CTCGTTTTGGATTTGGTTCTCAGAGTCTCGGGCAACGCCGAAGGTGATGCTCTAAGTGCA CTGAAAAACAGTTTAGCCGACCCTAATAAGGTGCTTCAAAGTTGGGATGCTACTCTTGTT ACTCCATGTACATGGTTTCATGTTACTTGCAATAGCGACAATAGTGTTACACGTGTTGAC CTTGGGAATGCAAATCTATCTGGACAGCTCGTAATGCAACTTGGTCAGCTTCCAAACTTG 15 CAGTACTTGGAGCTTTATAGCAATAACATTACTGGGACAATCCCAGAACAGCTTGGAAAT CTGACGGAATTGGTGAGCTTGGATCTTTACTTGAACAATTTAAGCGGGCCTATTCCATCA ACTCTCGGCCGACTTAAGAAACTCCGTTTCTTGCGTCTTAATAACAATAGCTTATCTGGA GAAATTCCAAGGTCTTTGACTGCTGTCCTGACGCTACAAGTTCTGGATCTCTCAAACAAT 20 CCTCTCACCGGAGATATTCCTGTTAATGGTTCCTTTTCACTTTTCACTCCAATCAGTTTT GCCAACACCAAGTTGACTCCCCTTCCTGCATCTCCACCGCCTCCTATCTCTCCTACACCG CCATCACCTGCAGGGAGTAATAGAATTACTGGAGCGATTGCGGGAGGAGTTGCTGCAGGT GCTGCACTTCTATTTGCTGTTCCGGCCATTGCACTAGCTTGGTGGCGAAGGAAAAAGCCG CAGGACCACTTCTTTGATGTACCAGCTGAAGAGGACCCAGAAGTTCATTTAGGACAACTG AAGAGGTTTTCATTGCGTGAACTACAAGTTGCTTCGGATAATTTTAGCAACAAGAACATA 25 TTGGGTAGAGGTGGTTTTGGTAAAGTTTATAAAGGACGGTTAGCTGATGGTACTTTAGTG GTTGAGATGATTAGTATGGCGGTTCACAGAAACTTGCTTCGGCTTCGTGGATTTTGCATG ACTCCAACCGAAAGATTGCTTGTTTATCCCTACATGGCTAATGGAAGTGTTGCCTCCTGT 30 TTAAGAGAACGTCCCGAGTCCCAGCCACCACTTGATTGGCCAAAGAGACAGCGTATTGCG TTGGGATCTGCAAGAGGGCTTGCGTATTTACATGATCATTGCGACCCAAAGATTATTCAT CGAGATGTGAAAGCTGCAAATATTTTGTTGGATGAAGAGTTTGAAGCCGTGGTTGGGGAT TTTGGACTTGCAAAACTCATGGACTACAAAGACACACATGTGACAACCGCAGTGCGTGGG ACAATTGGTCATATAGCCCCTGAGTACCTTTCCACTGGAAAATCATCAGAGAAAACCGAT 35 GTCTTTGGGTATGGAGTCATGCTTCTTGAGCTTATCACTGGACAAAGGGCTTTTGATCTT GCTCGCCTCGCGAATGATGATGATGTCATGTTACTAGACTGGGTGAAAGGGTTGTTAAAA GAGAAGAAATTGGAAGCACTAGTAGATGTTGATCTTCAGGGTAATTACAAAGACGAAGAA GTGGAGCAGCTAATCCAAGTGGCTTTACTCTGCACTCAGAGTTCACCAATGGAAAGACCC AAAATGTCTGAAGTTGTAAGAATGCTTGAAGGAGATGGTTTAGCTGAGAGATGGGAAGAG TGGCAAAAGGAGGAAATGTTCAGACAAGATTTCAACTACCCAACCCACCATCCAGCCGTG 40

5

15

20

25

Predicted amino acid sequence of the Arabidopsis thaliana RKS10 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the Cterminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30 MERRLMIPCFFWLILVL DLVLRVSGNAEG

DALSALKNSLADP NKVLQSWDATLVT

35

PCTWFHVTCNSDNSVTRV

 ${\tt DLGNANLSGQLV}$

M QLGQLPNLQYLELYSNNITGTI
40 PEQLGNLTELVSLDLYLNNLSGPI

WO 2004/007712 PCT/NL2003/000524 81

PSTLGRLKKLRFLRLNNNSLSGEI
PRSLTAVLTLQVLDLSNNPLTGDI
PVNGSFSLTPISFANTK LT PL

5 PASPPPPISPTPPSPAGSNRITG

AIAGGVAAGAAL LFAVPAIALAWW

10 RRKKPQDHFFDVPAEEDPE VHLGQLKRFSLRELQVAS

DNFSNKNILGRGGFGKVYKGRLAD
GTLVAVKRLKEERTQGGELQFQ

15 TEVEMISMAVHRNLLRLRGFCM
TPTERLLVYPYMANGSVASCLR
ERPESQPPLDWPKRQRIALGSA
RGLAYLHDHCDPKIIHRDVKAA
NILLDEEFEAVVGDFGLAKLMD

20 YKDTHVTTAVRGTIGHIAPEYL
STGKSSEKTDVFGYGVMLLELI
TGQRAFDLARLANDDDVMLLDW
VKGLLKEKKLEALVDVDLQGNY
KDEEVEQLIQVALLCTQSSPME

RPKMSEVVRMLE

GDGLAERWEEWQKEEMFRQDFNYPTHH

PAVSGWIIGDSTSQIENEYPSGPR

25

Arabidopsis thaliana RKS 11 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 ttgttaacctctcgtaactaaaatcttccATGGTAGTAACAAAGAAGACCATGAAGA TTCAAATTCATCTCCTTTACTCGTTCTTGTTCCTCTGTTTCTCTACTCTCACTCTATCTT CTGAGCCCAGAAACCCTGAAGTTGAGGCGTTGATAAGTATAAGGAACAATTTGCATGATC CTCATGGAGCTTTGAACAATTGGGACGAGTTTTCAGTTGATCCTTGTAGCTGGGCTATGA 15 GAGGTTTATCTGAGTCTATCGGAAATCTCACAAATCTCCGACAAGTGTCATTGCAAAATA ACAACATCTCCGGCAAAATTCCACCGGAGCTCGGTTTTCTACCCAAATTACAAACCTTGG ATCTTTCCAACAACCGATTCTCCGGTGACATCCCTGTTTCCATCGACCAGCTAAGCAGCC TTCAATATCTGAGACTCAACAACACTCTTTGTCTGGGCCCTTCCCTGCTTCTTTGTCCC AAATTCCTCACCTCTCTTGGACTTGTCTTACAACAATCTCAGTGGCCCTGTTCCTA 20 AATTCCCAGCAAGGACTTTAAACGTTGCTGGTAATCCTTTGATTTGTAGAAGCAACCCAC CTGAGATTTGTTCTGGATCAATCAATGCAAGTCCACTTTCTGTTTCTTTGAGCTCTTCAT CAGGACGCAGGTCTAATAGATTGGCAATAGCTCTTAGTGTAAGCCTTGGCTCTGTTGTTA TACTAGTCCTTGCTCTCGGGTCCTTTTGTTGGTACCGAAAGAACAAAGAAGGCTACTGA TCCTTAACTTAAACGCAGATAAACAAGAGGAAGGGCTTCAAGGACTTGGGAATCTAAGAA 25 GCTTCACATTCAGAGAACTCCATGTTTATACAGATGGTTTCAGTTCCAAGAACATTCTCG GCGCTGGTGGATTCGGTAATGTGTACAGAGGCAAGCTTGGAGATGGGACAATGGTGGCAG TGAAACGGTTGAAGGATATTAATGGAACCTCAGGGGATTCACAGTTTCGTATGGAGCTAG AGATGATTAGCTTAGCTGTTCATAAGAATCTGCTTCGGTTAATTGGTTATTGCGCAACTT CTGGTGAAAGGCTTCTTGTTTACCCTTACATGCCTAATGGAAGCGTCGCCTCTAAGCTTA 30 AATCTAAACCGGCATTGGACTGGAACATGAGGAAGAGGATAGCAATTGGTGCAGCGAGAG CTAATATTCTCTTAGACGAGTGCTTTGAAGCTGTTGTTGGTGACTTTGGACTCGCAAAGC TCCTTAACCATGCGGATTCTCATGTCACAACTGCGGTCCGTGGTACGGTTGGCCACATTG CACCTGAATATCTCCCACTGGTCAGTCTTCTGAGAAAACCGATGTGTTTGGGTTCGGTA 35 TACTATTGCTCGAGCTCATAACCGGACTGAGAGCTCTTGAGTTTGGTAAAACCGTTAGCC AGAAAGGAGCTATGCTTGAATGGGTGAGGAAATTACATGAAGAGATGAAAGTAGAGGAAC TATTGGATCGAGAACTCGGAACTAACTACGATAAGATTGAAGTTGGAGAGATGTTGCAAG TGGCTTTGCTATGCACACATATCTGCCAGCTCATCGTCCTAAAATGTCTGAAGTTGTTT TGATGCTTGAAGGCGATGGATTAGCCGAGAGATGGGCTGCTTCGCATAACCATTCACATT 40 TCTACCATGCCAATATCTCTTTCAAGACAATCTCTTCTGTCTACTACTTCTGTCTCAA

 $\label{eq:GCTTGACGCACATTGCAATGATCCAACTTATCAAATGTTTGGATCTTCGGCTTTCGATGATGACGATGATCATCAGCCTTTAGATTCCTTTGCCATGGAACTATCCGGTCCAAGA\underline{TAA}_{C}$ acaatgaaagaaagatatcatttttacgatggatcaaacaatccaatgaaaaaa

5

15

25

30

Predicted amino acid sequence of the Arabidopsis thaliana RKS11 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain

represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each

approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for 0-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular

domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single

leucine rich repeat, probably involved in protein / protein interactions.

MVVVTKKTMKIQIHLLYSFLFL

35 CFSTLTLSSEPRNPEV

EALISIRNNLHDP HGALNNWDEFSVD

PCSWAMITCSPDNLVIGL

GAPSQSLSGGLS

5 ESIGNLTNLRQVSLQNNNISGKI
PPELGFLPKLQTLDLSNNRFSGDI
PVSIDQLSSLQYLRLNNNSLSGPF
PASLSQIPHLSFLDLSYNNLSGPV
PKFPARTFNVAGNPLICRSN

10

PPEICSGSINASPL SVSLSSSSGRRSNR

LAIALSVSLGSVVIL

15 VLALGSFCWY

RKKQRRLLILNLNGADKQEE GLQGLGNLRSFTFRELHVYT

20 DGFSSKNILGAGGFGNVYRGKLGD

GTMVAVKRLKDINGTSGDSQFR

MELEMISLAVHKNLLRLIGYCA

TSGERLLVYPYMPNGSVASKLK

SKPALDWNMRKRIAIGAA

25 RGLLYLHEQCDPKIIHRDVKAA

NILLDECFEAVVGDFGLAKLLN

HADSHVTTAVRGTVGHIAPEYL

STGQSSEKTDVFGFGILLLELI

TGLRALEFGKTVSQKGAMLEW

30 VRKLHEEMKVEELLDRELGTNY

DKIEVGEMLQVALLCTQYLPAH

RPKMSEVVLMLE

GDGLAERWAASHNHSHFYHANI

35 SFKTISSLSTTSVSRLDAHCNDPTYQMFG

SSAFDDDDDHQPLDSFAMELSGPR

Arabidopsis thaliana RKS12 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- tttaaaaaccttgctagttctcaattctcatgactttgcttttagtcttagaagtggaaa ATGGAACATGGATCATCCCGTGGCTTTATTTGGCTGATTCTATTTCTCGATTTTGTTTCC AGAGTCACCGGAAAAACACAAGTTGATGCTCTCATTGCTCTAAGAAGCAGTTTATCATCA GGTGACCATACAAACAATATACTCCAAAGCTGGAATGCCACTCACGTTACTCCATGTTCA TGGTTTCATGTTACTTGCAATACTGAAAACAGTGTTACTCGTCTTGACCTGGGGAGTGCT AATCTATCTGGAGAACTGGTGCCACAGCTTGCTCAGCTTCCAAATTTGCAGTACTTGGAA 15 CTTTTTAACAATAATATTACTGGGGAGATACCTGAGGAGCTTGGCGACTTGATGGAACTA GTAAGCTTGGACCTTTTTGCAAACAACATAAGCGGTCCCATCCCTTCCTCTTGGCAAA CTAGGAAAACTCCGCTTCTTGCGTCTTTATAACAACAGCTTATCTGGAGAAATTCCAAGG ${\tt TCTTTGACTGCTCTGCCGCTGGATGTTCTTGATATCTCAAACAATCGGCTCAGTGGAGAT\\$ ATTCCTGTTAATGGTTCCTTTTCGCAGTTCACTTCTATGAGTTTTGCCAATAATAAATTA 20 AGGCCGCGACCTGCATCTCCTTCACCATCACCTTCAGGAACGTCTGCAGCAATAGTAGTG AAAAGGTTCTCCTTGCGTGAACTGCTAGTTGCTACAGAGAAATTTAGCAAAAGAAATGTA 25 TTGGGCAAAGGACGTTTTGGTATATTGTATAAAGGACGTTTAGCTGATGACACTCTAGTG GCTGTGAAACGCTAAATGAAGAACGTACCAAGGGTGGGGAACTGCAGTTTCAAACCGAA GTTGAGATGATCAGTATGGCCGTTCATAGGAACTTGCTTCGGCTTCGTGGCTTTTGCATG ACTCCAACTGAAAGATTACTTGTTTATCCCTACATGGCTAATGGAAGTGTTGCTTCTTGT 30 CTGGGATCAGCAAGGGGGCTCGCATATTTACACGATCATTGCGACCAAAAGATCATTCAC CTGGATGTGAAAGCTGCAAATATACTGTTAGATGAAGAGTTTGAAGCTGTTGTTGGAGAT TTTGGGCTAGCAAAATTAATGAATTATAACGACTCCCATGTGACAACTGCTGTACGGGGT ACGATTGGCCATATAGCGCCCGAGTACCTCTCGACAGGAAAATCTTCTGAGAAGACTGAT GTTTTTGGGTACGGGGTCATGCTTCTCGAGCTCATCACTGGACAAAAGGCTTTCGATCTT 35 GCTCGGCTTGCAAATGATGATGATATCATGTTACTCGACTGGGTGAAAGAGGTTTTGAAA GAGAAGAAGTTGGAAAGCCTTGTGGATGCAGAACTCGAAGGAAAGTACGTGGAAACAGAA GTGGAGCAGCTGATACAAATGGCTCTGCTCTGCACTCAAAGTTCTGCAATGGAACGTCCA AAGATGTCAGAAGTAGTGAGAATGCTGGAAGGAGATGGTTTAGCTGAGAGATGGGAAGAA TGGCAAAAGGAGGAGATGCCAATACATGATTTTAACTATCAAGCCTATCCTCATGCTGGC 40 ACTGACTGGCTCATCCCCTATTCCAATTCCCTTATCGAAAACGATTACCCCTCGGGGCCA

WO 2004/007712

PCT/NL2003/000524

86

5

Predicted amino acid sequence of the Arabidopsis thaliana RKS12 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain

represents a signal sequence. The second domain contains a leucine zipper motif, containing 2 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain

20 contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown

25. function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein

leucine rich repeat, probably involved in protein / protein interactions.

MEHGSSRGFI WLILFLDFVSRVTGKTQV

35

DALIALRSSLSSGDHTNNILQ SWNATHVT

PCSWFHVTCNTENSVTRL

DLGSANLSGELV

P QLAQLPNLQYLELFNNNITGEI

PEELGDLMELVSLDLFANNISGPI

PSSLGKLGKLRFLRLYNNSLSGEI

PRSLTALP LDVLDISNNRLSGDI

PVNGSFSQFTSMRFA NNKLRPR

10 PASPSPSGGTS

AAIVVGVAAGAALLFALAWWL

RRKLQGHFLDVPAAEEDPE

15 VYLGQFKRFSLRELLVAT

EKFSKRNVLGKGRFGILYKGRLAD
DTLVAVKRLNEERTKGGELQFQ
TEVEMISMAVHRNLLRLRGFCM

20 TPTERLLVYPYMANGSVASCLR
ERPEGNPALDWPKRKHIALGSA
RGLAYLHDHCDQKIIHLDVKAA
NILLDEEFEAVVGDFGLAKLMN
YNDSHVTTAVRGTIGHIAPEYL

25 STGKSSEKTDVFGYGVMLLELI
TGQKAFDLARLANDDDIMLLDW
VKEVLKEKKLESLVDAELEGKY
VETEVEQLIQMALLCTQSSAME

RPKMSEVVRMLE

30

GDGLAERWEEWQKEEMPIHDFNYQAY

PHAGTDWLIPYSNSLIENDYPSGPR

Arabidopsis thaliana RKS13 cDNA

The start codons encoding predicted the methionine residue of the gene product has been indicated by bold capitals. The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

 ${\tt taataaacctctaataataatggctttgcttttactctgatgacaagttcaaaa {\tt ATGGAA}}$ 10 ${\tt CAAAGATCACTCCTTTGCTTCCTTTATCTGCTCCTACTATTCAATTTCACTCTCAGAGTC}$ GCTGGAAACGCTGAAGGTGATGCTTTGACTCAGCTGAAAAACAGTTTGTCATCAGGTGAC CCTGCAAACAATGTACTCCAAAGCTGGGATGCTACTCTTGTTACTCCATGTACTTGGTTT CATGTTACTTGCAATCCTGAGAATAAAGTTACTCGTGTTGACCTTGGGAATGCAAAACTA TCTGGAAAGTTGGTTCCAGAACTTGGTCAGCTTTTAAACTTGCAGTACTTGGAGCTTTAT 15 ${\tt AGCAATAACATTACAGGGGAGATACCTGAGGAGCTTGGCGACTTGGTGGAACTAGTAAGC}$ TTGGATCTTTACGCAAACAGCATAAGCGGTCCCATCCCTTCGTCTCTTGGCAAACTAGGA AAACTCCGGTTCTTGCGTCTTAACAACAATAGCTTATCAGGGGAAATTCCAATGACTTTG ACTTCTGTGCAGCTGCAAGTTCTGGATATCTCAAACAATCGGCTCAGTGGAGATATTCCT GTTAATGGTTCTTTTCGCTCTTCACTCCTATCAGTTTTGCGAATAATAGCTTAACGGAT 20 $\tt CTTCCCGAACCTCCGCCTACTTCTACCTCCTACGCCACCACCACCTTCAGGGGGGGCAA$ ATGACTGCAGCAATAGCAGGGGGAGTTGCTGCAGGTGCAGCACTTCTATTTGCTGTTCCA GCCATTGCGTTGCTTGGTGGCTCAGAAGAAACCACAGGACCACTTTTTTGATGTACCT GCTGAAGAAGACCCAGAGGTTCATTTAGGACAACTCAAAAGGTTTACCTTGCGTGAACTG TTAGTTGCTACTGATAACTTTAGCAATAAAAATGTATTGGGTAGAGGTGGTTTTGGTAAA 25 GTGTATAAAGGACGTTTAGCCGATGGCAATCTAGTGGCTGTCAAAAGGCTAAAAGAAGAA CGTACCAAGGGTGGGGAACTGCAGTTTCAAACCGAAGTTGAGATGATCAGTATGGCCGTT CATAGGAACTTGCTTCGGCTTCGTGGCTTTTGCATGACTCCAACTGAAAGATTACTTGTT TATCCCTACATGGCTAATGGAAGTGTTGCTTCTTGTTTAAGAGAGCGTCCTGAAGGCAAT ${\tt CCAGCACTTGATTGGCCAAAAAGAAAGCATATTGCTCTGGGATCAGCAAGGGGGGCTTGCG}$ 30 TATTTACATGATCATTGCGACCAAAAAATCATTCACCGGGATGTTAAAGCTGCTAATATA TTGTTAGATGAAGAGTTTGAAGCTGTTGTTGGAGATTTTGGGCTCGCAAAATTAATGAAT TATAATGACTCCCATGTGACAACTGCTGTACGCGGTACAATTGGCCATATAGCGCCCGAG TACCTCTCGACAGGAAAATCTTCTGAGAAGACTGATGTTTTTGGGTACGGGGTCATGCTT CTCGAGCTCATCACTGGACAAAAGGCTTTCGATCTTGCTCGGCTTGCAAATGATGATGAT ATCATGTTACTCGACTGGGTGAAAGAGGTTTTGAAAGAGAAGAAGTTGGAAAGCCTTGTG 35 GATGCAGAACTCGAAGGAAAGTACGTGGAAACAGAAGTGGAGCAGCTGATACAAATGGCT CTGCTCTGCACTCAAAGTTCTGCAATGGAACGTCCAAAGATGTCAGAAGTAGTGAGAATG CTGGAAGGAGATGGTTTAGCTGAGAGATGGGAAGATGGCAAAAGGAGGAGATGCCAATA 40 ${\tt AATTCCCTTATCGAAAACGATTACCCCTCGGGTCCAAGA\underline{TAA}{\tt ccttttagaaagggtctt}}$

ttcttgtgggttcttcaacaagtatatatatatagattggtgaagttttaagatgcaaaaaa

5

10

Predicted amino acid sequence of the Arabidopsis thaliana RKS13 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt $et\ al.\ (1997)$.

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains leucine zipper motifs, containing 2 times 2 leucine residues, each separated by seven other amino acids. The third domain

contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to

contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably

also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30

MEQRSLLCFLYLL LLFNFTLRVAGNAEG

35 DALTQLKNSLSSGDP ANNVLQSWDATLVT

PCTWFHVTCNPENKVTRV

DLGNAKLSGKLV
P ELGQLLNLQYLELYSNNITGEI
PEELGDLVELVSLDLYANSISGPI
5 PSSLGKLGKLRFLRLNNNSLSGEI
PMTLTSVQLQV LDISNNRLSGDI
PVNGSFSLFTPISFANNSLTDLPE

PPPTSTSPTPPPPSG

10

GQMTAAIAGGVAAGAAL LFAVPAIAFAWWL

RRKPQDHFFDVPGAEEDPE

15 VHLGQLKRFTLRELLVAT

DNFSNKNVLGRGGFGKVYKGRLAD
GNLVAVKRLKEERTKGGELQFQ
TEVEMISMAVHRNLLRLRGFCM

20 TPTERLLVYPYMANGSVASCLR
ERPEGNPALDWPKRKHIALGSA
RGLAYLHDHCDQKIIHRDVKAA
NILLDEEFEAVVGDFGLAKLMN
YNDSHVTTAVRGTIGHIAPEYL

25 STGKSSEKTDVFGYGVMLLELI
TGQKAFDLARLANDDDIMLLDW
VKEVLKEKKLESLVDAELEGKY
VETEVEQLIQMALLCTQSSAME

30

GDGLAERWEEWQKEEMPIHDFNYQA

RPKMSEVVRMLE

YPHAGTDWLIPYSNSLIENDYPSGPR

Arabidopsis thaliana RKS14 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 ctgcaccttagagattaatactctcaagaaaaacaagttttgattcggacaaagATGTTG CAAGGAAGAAGAAGAAAAAAGAGTTATGCTTTGTTCTTCTAACTTTCTTCTTCTTC TTTATCTGTTTTCTTCTTCTTCTTCTGCAGAACTCACAGACAAGTTGTTGCCTTAATA GGAATCAAAAGCTCACTGACTGATCCTCATGGAGTTCTAATGAATTGGGATGACACAGCA GTTGATCCATGTAGCTGGAACATGATCACTTGTTCTGATGGTTTTGTCATAAGGCTAGAA 15 GCTCCAAGCCAAAACTTATCAGGAACTCTTTCATCAAGTATTGGAAATTTAACAAATCTT CAAACTGTATACAGGTTATTGCAGAACAATTACATAACAGGAAACATCCCTCATGAGATT GGGAAATTGATGAAACTCAAAACACTTGATCTCTCTACCAATAACTTCACTGGTCAAATC CCATTCACTCTTTCTTACTCCAAAAATCTTCACAGGAGGGTTAATAATAACAGCCTGACA GGAACAATTCCTAGCTCATTGGCAAACATGACCCAACTCACTTTTTTTGGATTTGTCGTAT 20 AATAACTTGAGTGGACCAGTTCCAAGATCACTTGCCAAAACATTCAATGTTATGGGCAAT TCTCAGATTTGTCCAACAGGAACTGAGAAAGACTGTAATGGGACTCAGCCTAAGCCAATG TCAATCACCTTGAACAGTTCTCAAAGAACTAAAAACCGGAAAATCGCGGTAGTCTTCGGT GTAAGCTTGACATGTGTTTGCTTGTTGATCATTGGCTTTGGTTTTCTTCTTTGGTGGAGA AGAAGACATAACAAACAAGTATTATTCTTTGACATTAATGAGCAAAACAAGGAAGAAATG 25 TGTCTAGGGAATCTAAGGAGGTTTAATTTCAAAGAACTTCAATCCGCAACTAGTAACTTC AGCAGCAAGAATCTGGTCGGAAAAGGAGGGTTTGGAAATGTGTATAAAGGTTGTCTTCAT GATGGAAGTATCATCGCGGTGAAGAGATTAAAGGATATAAACAATGGTGGTGGAGAGGTT CAGTTTCAGACAGAGCTTGAAATGATAAGCCTTGCCGTCCACCGGAATCTCCTCCGCTTA TACGGTTTCTGTACTACTTCCTCTGAACGGCTTCTCGTTTATCCTTACATGTCCAATGGC 30 AGTGTCGCTTCTCGTCTCAAAGCTAAACCGGTATTGGATTGGGCCACAAGAAAGCGAATA GCATTAGGAGCAGGAAGAGGGTTGCTGTATTTGCATGAGCAATGTGATCCAAAGATCATT CACCGTGATGTCAAAGCTGCGAACATACTTCTTGACGATTACTTTGAAGCTGTTGTCGGA GATTTCGGGTTGGCTAAGCTTTTGGATCATGAGGAGTCGCATGTGACAACCGCCGTGAGA GGAACAGTGGGTCACATTGCACCTGAGTATCTCTCAACAGGACAATCTTCTGAGAAGACA 35 GATGTGTTCGGTTTCGGGATTCTTCTCCGAATTGATTACTGGATTGAGAGCTCTTGAA TTCGGAAAAGCAGCAAACCAAAGAGGGGGGGGTACTTGGTTAGAGAAACTACAACAA GAGAAGAAGCTAGAACAGATAGTAGACAAGGATTTGAAGAGCAACTACGATAGAATAGAA GTGGAAGAAATGGTTCAAGTGGCTTTGCTTTGTACACAGTATCTTCCCATTCACCGTCCT AAGATGTCTGAAGTTGTGAGAATGCTTGAAGGCGATGGTCTTGTTGAGAAATGGGAAGCT 40 TCTTCTCAGAGAGCAGAAACCAATAGAAGTTACAGTAAACCTAACGAGTTTTCTTCCTCT

 ${\tt GAACGTTATTCGGATCTTACAGATGATTCCTCGGTGCTGGTTCAAGCCATGGAGTTATCA}$

 ${\tt GGTCCAAGATGAcaagagaaactatatgaatggctttgggtttgtaaaaaa}$

5

10

15

20

25

30

Predicted amino acid sequence of the Arabidopsis thaliana RKS14 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for Oglycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine

protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

MLQGRREAKKSYALFSSTFF **FFFICFLSSSSAELTDKV**

35 VALIGIKSSLTDP **HGVLMNWDDTAVD**

PCSWNMITCSDGFVIR

LEAPSQNLSGTLSS

SIGNLTNLQTVYRLLQNNYITGNI PHEIGKLMKLKTLDLSTNNFTGQI

PFTLSYSKNLHRRV NNNSLTGTI

PSSLANMTQLTFLDLSYNNLSGPV

PRSLAKTFNVMGNSQICPT

GTEKDCNGTQPKPMSITLNSSQR

10 TKNRK

5

IAVVFGVSLTCVCLLIIGFGFLLWW

RRRHNKQVLFFDINEQNKE

15 EMCLGNLRRFNFKELQSAT

SNFSSKNLVGKGGFGNVYKGCLHD

GSIIAVKRLKDINNGGGEVQFQ

TELEMISLAVHRNLLRLYGFCT

20 TSSERLLVYPYMSNGSVA

SRLKAKPVLDWGTRKRIALGAG

RGLLYLHEQCDPKIIHRDVKAA

NILLDDYFEAVVGDFGLAKLLD

HEESHVTTAVRGTVGHIAPEYL

25 STGQSSEKTDVFGFGILLLELI

TGLRALEFGKAANQRGAILDW

VKKLQQEKKLEQIVDKDLKSNY

DRIEVEEMVQVALLCTQYLPIH

RPKMSEVVRMLE

30

GDGLVEKWEASSQRAET

NRSYSKPNEFSSS

ERYSDLTDDSSVLVQAMELSGPR

Legends

Figure 1

5

The different domains of the predicted RKS gene product have the following functions: The first domain of the predicted protein structure at the Nterminal end consists of a signal sequence, involved in targeting the protein towards the plasma membrane. Protein 10 cleavage removes this sequence from the final mature protein product (Jain et al. 1994, J. Biol. Chemistry 269: 16306-16310). The second domain consists of different numbers of leucine zipper motifs, and is likely to be involved in protein 15 protein dimerization. The next domain contains a conserved pair of cystein residues, involved in disulphate bridge formation. The next domain consists of 5 (or in the case of RKS3 only 4) leucine rich repeats (LRRs) shown in a gray colour, likely to be involved in ligand binding (Kobe and Deisenhofer 1994, TIBS 19: 415-420). This domain is again 20 bordered by a domain containing a conserved pair of cystein residues involved in disulphate bridge formation often followed by a serine /. proline rich region. The next domain displays all the characteristics of a single transmembrane 25 domain (http://genome.cbs.dtu.dk/services/TMHMM/). At the predicted cytoplasmic site of protein a domain is situated with unknown function, followed by a domain with serine /threonine kinase activity (Schmidt et al. 1997, Development 124: 2049-2062). The kinase domain is followed by a domain 30 with unknown function whereas at the C-terminal end of the protein part of a leucine rich repeat is positioned, probably involved in protein-protein interactions.

Figure 2

Alagnment of the predicted protein sequences of the different RKS gene products from Arabidopsis thaliana with alignX,
Vector NTI Suite 5.5 resulted in a phylogenetic tree in which

the relative homology between the different RKS members is shown.

Figure 3

Intron-Exon bounderies of the genomic regions on the chromosomes of Arabidopsis thaliana encoding the different RKS gene products. Exons are shown as boxes, whereas intron sequences are shown as lines. Sequences encoding LRR domains are displayed in gray colour, transmembrane regions in black.

10

Figure 4.

Cromosomal location of RKS genes in Arabidopsis thaliana, showing colocalisation with GASA genes.

Figure 5. A signaling complex comprising molecules of RKS proteins, ELS proteins, NDR/NHL proteins and SBP/SPL proteins.

Figure 6.

- Second generation (T2) tobacco seedlings germinated on MS
 medium. Transformations were performed with DNA clone 2212-15,
 representing the overexpression construct GT-RKS4-s. T2
 seedlings derived from T1 plant 15.7 shows co-suppression
 effects while T1 plant 15.6 shows no obvious changes in level
 of RKS4. T1 plants 15.9 and 15.3 show overexpression effects.
- 25 Plant 15.7 has the lowest remaining level of RKS4 gene product, whereas plant 15.3 has the highest level of RKS4 gene product.

Figure 7

- 30 Second generation (T2) tobacco plants. In the upper row the offspring from a co-suppressing T1 plant 15.7 is shown. The middle row shows plants derived from a transgenic T1 plant 15.6 with no clear changes in level of RKS4 is shown while the bottom row shows plants derived from a T1 plant 15.3 in which 35 the levels of RKS4 are increased by the introduction of the
- 35 the levels of RKS4 are increased by the introduction of the overexpression construct GT-RKS4-s.

Figure 8

Second generation (T2) tobacco plants. Plants derived from a co-suppressing T1 plant 15.7 show a reduction in plant size and a delay in the initiation and outgrowth of primordia. The control empty vector transgenic plants show no visible differences in growth compared with the offspring from the transgenic 15.6 plant, in which the endogenous level of RKS4 gene product was not changed. In the overexpressing plants 15.9 and 15.3 organ size was increased, similar as the number of initiated leaf primordia.

Figure 9

10

15

20

25

30

35

Arabidopsis thaliana WS plants in which the endogenous level of RKS4 gene product is decreased (right picture) due to the presence of a transgenic RKS4 antisense construct (GT-RKS4-16a). The left picture shows a wildtype plant of the same age as the transgenic antisense plant, grown under similar growth conditions. Plant size, organ size and number of organ primordia is decreased in the transgenic antisense plant compared with the wildtype control.

Figure 10.

Arabidopsis thaliana WS plants in which the endogenous level of RKS4 gene product is decreased (bottom left picture) due to the presence of a transgenic RKS4 antisense construct (GT-RKS4-16a). The upper right picture shows a wildtype flower of the same age as the transgenic antisense flower, grown under similar growth conditions. Total flower size is only slightly decreased in the transgenic antisense flower compared with the control flower, whereas organ size of petals is strongly decreased.

Arabidopsis thaliana WS plants in which the endogenous level of RKS4 gene product is increased (upper left picture) due to the presence of a transgenic RKS4 overexpressing construct (GT-RKS4-6s). Compared with the wildtype control flower, total flower size of the transgenic flower is clearly increased. Both sepal and petal organ size is clearly increased compared

with the control.

For comparison an Arabidopsis thaliana WS plant is shown which has been transformed with a construct encoding the GASA3 gene in sense direction, i.e. overexpressing GASA3.

5

Figure 11.

Formation of meristematic regions in the hypocotyl of Arabidopsis thaliana WS plants under influence of overexpression of RKS4.

10 RKS4 overexpression results in increases in flower and seed organ size that could be due to increase in cell elongation and/or cell division. In order to analyse the cell division patterns in plants with deregulated RKS4 expression the mitotic activity in transgenic plants was analyzed with the a unstable GUS reporter under the control of a cyclin B1;1 15 promoter (the Plant Journal 1999 (4) 503-508 Spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein). Arabidopsis thaliana WS seedlings with the pCDG construct did not show gus activity (cell division) in hypocotyls (top) whereas the same pCDG line crossed with a 20 constitutive RKS4 construct showed mitotic activity as indicated by GUS-positive cells (bottom); indicating that RKS4 overexpression activated mitotic activity in hypocotyls.

25 Figure 12

In Arabidopsis thaliana WS, the seed size is influenced by changing levels of RKS4 gene product. Constitutive overexpression of RKS4 results in increases in seed size (left) compared with control wildtype seeds (right). Antisense constitutive expression of RKS4 cDNA (middle) results in a decrease in seed size compared with the control (right). Magnification is identical in all photos as shown by the bar size.

35

30

Organ size can be influenced by either modulating cell division or cell elongation or a combination of both. In order to identify the total number of cells and the cell size within an organ the apical site of petals of mature Arabidopsis flowers was investigated. Petal organ size is clearly influenced by modulation of RKS4 gene product levels (bottom row for the flowers from which the apical petal epidermal cells were identified). Epidermal cell size is not changed in transgenic plants compared with the control.

10

Figure 14

Arabidopsis thaliana WS plants in which the endogenous level of RKS10 gene product is increased (right picture) due to the presence of a transgenic RKS10 overexpressing construct. The left picture shows the apical epidermus of a full grown cotyl from an empty vector transgenic seedling of the same age as the transgenic overexpressing cotyl, grown under similar growth conditions.

20 Figure 15

Arabidopsis thaliana WS plants in which the endogenous level of RKS10 gene product is decreased (right picture) due to the presence of a RKS10 antisense construct The left picture shows a wildtype plant of the same age as the transgenic antisense plant, grown under similar growth conditions. Plant size, organ size and number of organ primordia remains similar in both the transgenic antisense plants and the wildtype control.

30 Figure 16

In order to determine organ size variations in transgenic RKS10 transgenic plants compared with empty vector control transgenic plants (pGreen4K), flower organ size was determined of the four open flower stages of *Arabidopsis* inflorescences.

35 The four successive flower stages are photographed under similar magnifications. No obvious changes in organ length could be observed in size of sepals, petals, stamen and carpel

between empty vector control flowers (pGreen4K), flowers with an antisense RKS10 construct (a) or plants overexpressing the RKS10 cDNA under the control of a 35S promoter (S

5 Figure 17

Tissue cultured auxin treated transgenic Arabidopsis T2 seedlings were grown on MS agar plates without hormones for a period of 3 weeks. Regeneration potential was scored and the formation and outgrowth of multiple shoot apical meristems

- from single seedling origin was displayed as (+). The formation and outgrowth of only one shoot apical meristem, leading to the formation of a normal rosette of leaves from individual plants was displayed as (-). Positive regeneration controls consisted of seedlings overexpressing either KNAT1,
- 15 CUC2, IPT or cycD3. All of these showed an increase of regeneration capacity (+) compared with a negative control GUS overexpressing plant pGreen5K (-).

Representative examples of RKS and ELS cDNA overexpressing (s) or antisense (a) cosuppressing constructs in transgenic plants are shown in the bottom panels.

Figure 18.

20

Tobacco leaf discs were stably transformed with the RKSO overexpressing construct GT-RKSO-23S and from a single transformation event, large numbers of regeneration plantlets were isolated and subcultured. All of the regenerated plants were potted and flowered. The original transformation event could be kept continuously in tissue culture indefinitely.

30 Figure 19

Seedlings from transgenic Arabidopsis thaliana containing either constructs overexpressing (s) or co-suppressing by antisense (a) the RKS gene products were screened for the appearance of fasciation. Several examples in which fasciation could be routinely observed are shown together with a negative control plant (pGreen5K, overexpressing the GUS gene) in which fasciation could never be observed.

Figure 20 - 23

Primary root tips of transgenic Arabidopsis plants (top rows) photographed under similar magnification. The bottom rows show the corresponding seedlings (also between each other under the same magnification). Figure 23 shows the specific Arabidopsis transgenes with a strong increase in root outgrowth.

Figure 24

10 Avarage root length of 10-30 transgenic Arabidopsis T2 seedlings from one T1 transgenic plant is shown.

Figure 25

T3 seedlings are shown from a strong co-suppressing RKS10

15 antisense construct line (T1-4; T2-6; T3 generation) and a strong overexpressing line (T1-4; T2-6; T3 generation). The overexpressing line is different and stronger from the one shown in Figure 4.1-4.5. Pictures are taken under similar magnifications.

20

25

30

Figure 26

T2 seed was germinated on horizontal MS agar plates and pictures were taken under similar magnification of representative examples of the lateral root development from transgenic RKS and ELS transgenic roots.

Figure 27

Pictures taken from transgenic RKS8 or RKS10 overexpressing roots taken directly behind the tip zone. Pictures are taken under same magnification.

Figure 28

Arabidopsis thaliana WS plants in which the endogenous level of RKS or ELS gene product is modified result in the formation of new meristem formation and / or outgrowth, resulting in a complex, bushy inflorescence in transgenic Arabidopsis plants compared with control empty vector control plants (pGreen4K).

101

Overexpression of RKS10 and ELS1 (S) and cosuppression with antisense constructs of RKS8 and also RKS10, result in increased numbers of developing generative meristems. The generative shoots are photographed with similar magnification.

Figure 29

Arabidopsis thaliana WS plants in which the endogenous level of RKS gene product is modified result in the formation of new 10 meristem formation and / or outgrowth, resulting in a complex, bushy inflorescence in transgenic Arabidopsis plants commpared with control empty vector control plants (pGreen4K). The top panel shows adult plants under similar magnification. Compared with the control, RKS10 overexpression results in an extreme 15 bushy phenotypic plant. The results of co-suppressing the RKS8 gene product are less dramatic with respect to the bushiness. However, also in these transgenic plants the number of generative meristems is strongly increased compared with the control. The bottom panel shows the generative shoot in detail 20 under similar magnification.

Figure 30

25

Schematic drawing of the different flower organs in an empty vector control pGreen4K flower (left) compared with a complex transgenic flower structure seen in transgenic Arabidopsis plants containing an antisense (a) RKS10 construct. The terminal flower meristem produces 2 sepals, 1 petal, 2 stamen, a carpel which is not a closed structure but open with groups of ovules on the inside and outside of this structure, and stigmatic cells protruding from the top part. Two new flowers are protruding from this structure, containing all flower organs in normal numbers.

Figure 31

35 Schematic drawing of the different flower organs in a complex transgenic flower structure seen in transgenic Arabidopsis plants T1-11 containing an antisense (a) RKS10 construct. The

terminal flower meristem produces 1 sepal, 2 petals, 2 stamen, a carpel which is not a closed structure but open with groups of ovules on the inside and outside of this structure, and stigmatic cells protruding from the top part. An undetermined 5 flower meristem is protruding from the open carpel structure and forms a number of new flowers, including normal flowers (right) and another abnormal flower (left) which consists of a flower with half of the sepal, petal and stamen organs formed and a new terminal flower meristem protruding from this structure, developing in structures as seen in Figure 7.5. The stamen contain only small numbers of (viable) pollen compared with wildtype stamen (see also chapter 5).

Figure 32

10

Schematic drawing of the different flower organs in an empty 15 vector control pGreen4K flower (left) compared with a complex transgenic flower structure seen in a transgenic Arabidopsis plant T1-11 containing an antisense (a) RKS10 construct (overview shown in Figure 7.4). The terminal flower meristem produces half the normal number of sepals, petals and stamen. 20 The remaining part of the flower structure has converted into a new structure containing a new stem containing a single organ structure resembling a fusion between a petal and a sepal. On this structure several (viable) pollen grains can be 25 observed.

Figure 33

30

Schematic drawing of the different flower organs in a complex transgenic flower structure seen in a transgenic Arabidopsis plant T1-12 containing an antisense (a) RKS10 construct. The terminal flower meristem originating from an undetermined generative meristem is here producing an axillary secondary undetermined meristem (left picture), a single organ resembling a stamen (bottom left), a normal flower and a terminal flower. This terminal flower structure contains 2 normal sepals, 2 normal petals, 2 normal stamen (with only a few viable pollen) and two organs resembling a fusion of

sepals /petals/stamen (see also figure 7.7). From this terminal flower structure two new flowers emerge (in a similar fashion as observed in Figure 7.3) containing normal numbers of flower organs (right photos). At the top of this figure a control inflorescense is shown schematically with terminal flower meristems as normally originate from the generative Arabidopsis thaliana generative meristem.

Figure 34

Schematic drawing and detailed pictures of several of the structures as shown in figure 7.6. At the right the organs resembling a fusion between sepals/petals/stamen are shown with viable pollen sticking out from these structures. At the top left the single stamen-like organ directly protruding from the main stem is shown.

Figure 35

20

25

30

35

Transgenic Arabidopsis plants overexpressing the RKS13 gene product show a modification of the normal flower inflorescence architecture, somewhat resembling the structures observed in RKS10 antisense plants. A terminal flower containing a normal seed developing silique and a small number of sepals, petals and stamen, develops at least 4 additional terminal flower meristems that develop abnormally themselves, resulting in open carpel structures and modifications of organ structures.

Figure 36

Transgenic plants in which the RKS and / or ELS genes are introduced behind a constitutive 35S promoter in an overexpressing (S) or antisense (a) configuration are analyzed for sterility and characterized further for defects in proper pollen development. As a negative control the normal pollen development of a transgene containing the empty expression vector (pG4K) was included. First generation transgenic flowers of RKS1O expressing constructs and second generation control vector and ELS2 are shown under similar magnification. In detail the stigmatic surface and surrounding stamen, are

WO 2004/007712 PCT/NL2003/000524 104

shown under similar magnification, showing the presence or absence of pollen on the stamen or the stigmatic surface.

Detailed description

1. Modifying organ size

5

10

15

20

30

the total plant size

Plant size is determined by both cell elongation and cell division rate. Modifying either one or both processes results in a change in final organ size. Increasing the level of specific members of the family of RKS genes results in an increase in organ size, growth rate and yield. Modulating plant growth, organ size and yield of plant organs is the most important process to be optimized in plant performance. Here we show that modulating the level of members of the family of the RKS signaling complex is sufficient to modulate these processes. The invention provides herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKJS 10 gene or functional equivalent thereof. Inactivation of endogenous RKS gene product results in a decrease in plant growth, proving that the normal function of these endogenous RKS gene products is the regulation of growth and organ size. Elevation of the levels of the regulating of the RKS signaling complex in plant cells is provided in order to increase: the size of plant organs the growth rate the yield of harvested crop the yield of total plant material

35 Decreasing the levels of endogenous RKS gene product is provided in order to decrease:

the size of plant organs

the growth rate the total plant size

5

Results obtained (see also figures 6 to 13)

Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in Arabidopsis thaliana and in Nicotiana tabacum. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail. The phenotype observed in transgenic plants with antisense constructs of RKS4 (GT-RKS4-a) could be described as dwarf plants in which all plant organs showed a decrease in organs size and growth rate. Overexpression of RKS4 (GT-RKS4-s) resulted in plants with increased size of organs and an increase in growth rate Since cell size alone was not responsible for the modifications in organ size of petals it can be concluded that RKS4 is involved in the regulation of the cellular divisions during plant growth and organ formation. Overexpression of RKS 4 results in an increase of cellular divisions whereas a decrease in endogenous RKS 4 gene product levels within the plant results in a decrease of cellular division rates.

25

15

Literature

- -Not being the wrong size. R.H. Gomer 2001; Nature reviews 2: 48-54
- -Cell cycling and cell enlargement in developing leaves of

 30 Arabidopsis. P.M Donnelly et al. 1999; Developmental biology
 215: 407-419
 - -Ectopic expression of A. integumenta in Arabidopsis plants results in increased growth of floral organs. B.A. Krizek 1999 Developmental genetics 25: 224-236
- 35 -Plant organ size control: A. integumenta regulates growth and cell numbers during organogenesis. Y. Mizukami and R.L. Fischer PNAS 97: 942-947

-Measuring dimensions: the regulation of size and shape. S.J. Day and P.A.Lawrence 2000; Development 127: 2977-2987

-A matter of size: developmental control of organ size in plants. Y. Mizukami 2001; Current opinions in plant biology 4: 533-539

2. Cell division

The mitotic cell cycle in eukaryotes determines the total number of cells within the organism and the number of cells within individual organs. The links between cell proliferation, cell differentiation and cell-cycle machinery are of primary importance for eukaryotes, and regulation of these processes allows modifications during every single stage of development. Here we show that modulating the level of members of the family of the RKS signaling complex is 10 sufficient to modulate these processes. The invention provides herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a 15 protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKJS 10 gene or functional 20 equivalent Herewith the invention provides a method for modulating the number of cells to be formed within an eukaryotic organism as a whole or for modulating the cell number within individual organs is, which of primary importance in modulating plant developmental processes, 25 especially of arable plants. Here we show that members of the RKS signaling complex are able to regulate the number of cellular divisions, thereby regulating the total number of cells within the organism or different organs.

30 Possible Applications

35

Elevation of the levels of the regulating RKS signaling complex members in plant cells in order to increase: the size of plant organs the growth rate the yield of harvested crop the yield of total plant material the total plant size

Decreasing the levels of endogenous RKS signaling complex members in order to decrease: the size of plant organs

5 the growth rate the total plant size

Results obtained

25

30

cellular division.

Overexpression and antisense constructs of full length RKS

10 cDNA clones have been made under the control of 35S

promoters. Transgenic plants have been produced in Arabidopsis

thaliana and in Nicotiana tabacum. Subsequent generations of

stably transformed plants were investigated for phenotypes and
analyzed in detail.

- Overexpression of RKS 4 results in an increase of cellular divisions whereas a decrease in endogenous RKS 4 gene product levels within the plant results in a decrease of cellular division. Another example of RKS genes involved in cellular proliferation is provided by RKS10. Overexpression of RKS10
- 20 (S) results in a decrease in apical epidermal cells (Figure 14) compared with control plants containing an empty expression cassette (pGreen4K). Co-suppressing the endogenous RKS 10 gene in plants containing an antisense construct (a) showed clearly larger epidermal cells as the corresponding
 - cells in wildtype control plants (Figure 15). In contrast to the plant phenotypes shown in RKS4 transgenic plants, no differences in plant or organ size could be observed in the RKS10 transgenic plants or organs. This shows that although the organ size remains constant, the number of cells within these organs is variable due to the differences in size of individual cells. These results indicate that normal RKS4

function within the plant can be described as an activator of

Normal RKS10 function also involves an activation process on cellular division rate. This effect is also detectable in the root in the region directly behind the tip zone, where in the RKS10 overexpressing transgenes cellular divisions were

detectable in a region where normally cell proliferation has ceased. The plane of divisions of root cells in these transgenes is also clearly different from the normal plane of root cell division, resulting in clumps of cells with all 5 types of division planes possible.

In contrast to RKS4, the final organ size in RKS10 transgenic plants is under the control of other organ size restriction processes, in such a way that the final organ volume remains constant (Figure 16). RKS4 and RKS10 are essentially involved in the same cell cycle activation process, but either addition organ size controlling functions of these RKS genes or the hierarchical order in which they regulate the cell cycle is

15

20

10

Literature

different.

-Not being the wrong size. R.H. Gomer 2001; Nature reviews 2: 48-54

-Cell cycling and cell enlargement in developing leaves of Arabidopsis. P.M Donnelly et al. 1999; Developmental biology 215: 407-419

-When plant cells decide to divide. H. Stals and D. Inze 2001.

Trends in Plant Science 6: 359-363

-Cell cycling and cell enlargement in developing leaves of Arabidopsis. P.M. Donnelly et al. 1999. Developmental Biology 215: 407-419

-Triggering the cell cycle in plants. B.G.W. den Boer and J.A.H. Murray 2000. Trends in Cell Biology 10: 245-250 30

3. Regeneration

Modification the levels of different RKS and ELS genes within plants allows the initiation and / or outgrowth of apical meristems, resulting in the formation of large numbers of plantlets from a single source. A number of gene products that is able to increase the regeneration potential of plants is known already. Examples of these are KNAT1, cycD3, CUC2 and 10 IPT. Here we show that modulation of the endogenous levels of RKS genes results in the formation of new shoots and plantlets in different plant species like Nicotiana tabacum and Arabidopsis thaliana. herewith the invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of 15 said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating apical meristem formation, in particular 20 wherein said gene comprises an ELS1, RKS0, RKS3, RKS4, RKS8 or RKS10 gene or functional equivalent thereof. A direct application of a method according to the invention is the stable or transient expression of RKS and ELS genes or gene products in order to initiate vegetative reproduction. 25 Regeneration can be induced after overexpression of for example RKSO and ELS1; or by co-suppression of for example the endogenous RKS3, RKS4, RKS8 or RKS10 genes. Overexpression or co-suppression of these RKS and ELS gene products can be either transient, or stable by integration of the 30 corresponding expression cassettes in the plant genome.

Results obtained

35

Overexpression and antisense constructs of full length RKS and ELS cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in Arabidopsis thaliana and in Nicotiana tabacum. Subsequent generations of

stably transformed plants were investigated for phenotypes and analyzed in detail.

T2 transgenic seedlings of Arabidopsis were germinated in liquid MS medium supplemented with 1 mg/L 2,4-D for 1 week, followed by extensive washing and plating of the seedlings onto MS agar plates without hormones. Control transgenic seedstocks containing either a negative control vector (pGreen5K); or positive control overexpression constructs of gene products known to increase the regeneration potential 10 (IPT, KNAT1, CUC2 and cycD3) were characterized for regeneration potential together with seedstocks from plants either overexpressing (s) or co-suppressing (a) all RKS and ELS gene products (Figure 17). Overexpression of the ELS1 and RKSO cDNA clones resulted in an increase of shoot apical meristem formation and outgrowth, whereas antisense constructs (a) of these cDNA clones did not increase the regeneration potential (only increased regeneration results are shown). Antisense constructs of RKS3, RKS4, RKS8 and RKS10 also resulted in an increased formation and outgrowth of apical 20 meristems (Figure 17).

T1 generation Nicotiana tabacum tissue cultures transformed with ELS and RKS gene products in either overexpression (s) cassettes or antisense co-suppression (a) cassettes allowed the regeneration of indefinite number of offspring plants from a single transformed cell origin (Figure 18). An example is shown for the overexpression of the GT-RKSO-23S construct. The resulting plants obtained from one transformation event in general showed no phenotypes. Only a subset of plants displayed RKSO overexpression phenotypes (like loss of apical dominance and early flowering).

Literature

25

30

-Mechanisms that control knox gene expression in the 35 Arabidopsis shoot. N. Ori et al. 2000, Development 127: 5523-5532

-Overexpression of KNAT1 in lettuce shifts leaf determinate growth to a shoot-like indeterminate growth associated with an accumulation of isopentenyltype cytokinins. G. Frugis et al. 2001. Plant Physiology 126: 1370-1380

- 5 -KNAT1 induces lobed leaves with ectopic meristems when overexpressed in *Arabidopsis*. Chuck et al. 1996. the Plant Cell 8: 1277-1289
 - -Cytokinin activation of Arabidopsis cell division through a D-type cyclin. C. Riou-Khamlichi et al. 1999. Science 283:
- 10 1541-1544

4. Fasciation

Fasciation is normally a result from an increased size of the apical meristem in apical plant organs. Modulation of the number of cells within the proliferating zone of the shoot apical meristem results in an excess number of cellular divisions, giving rise to excess numbers of primordia formed or to stems in which the number of cells is increased. The invention herewith provides a method for 10 modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing 15 modulating fasciation, in particular wherein said gene comprises an RKSO, RKS3, RKS8 or RKS10 gene or functional equivalent thereof. Here we for example show that modulation of the levels of RKS gene products in plants like Arabidopsis thaliana can result in fasciated stems as shown in Figure 19. 20 A direct application as provided herein is the regulated formation of fasciation in plant species in which such a trait is desired like ornamental plants. Regulation of the initiation and extent of fasciation, either by placing the responsible RKS encoding DNA sequences under the control of 25 stage or tissue specific promoters, constitutive promoters or inducible promoters results in plants with localized or constitutive fasciation of stem tissue. Another application is modulating the number of primordiae by regulation of the process of fasciation. An example is provided by for example 30 sprouts, in which an increased number of primordia will result in an increased numbers of sprouts to be harvested. Fasciation can also result in a strong modification in the structural architecture of the inflorescence, resulting in a terminal group of flowers resembling the Umbelliferae type (an example 35 is shown in Figure 19 where the fasciated meristem of a RKSO-

7S Arabidopsis plant in which endogenous RKSO gene product

114

levels have been deregulated clearly terminates in an Umbelliferae type inflorescence.

Results obtained

- Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in *Arabidopsis thaliana*. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail.
- 10 T2 transgenic seedlings of Arabidopsis were germinated on MS agar plates without hormones. Control transgenic seedstocks containing a negative control vector (pGreen5K) were tested for their ability to induce fasciation (Overexpression constructs (s) of RKSO, RKS8 and RKS10 cDNA clones resulted in
- fasciated plants, whereas antisense constructs (a) of these cDNA clones did not increase the regeneration potential (only positive results are shown). Antisense constructs of RKS3 gave also rise to fasciation (Figure 19).

20

Literature

- -Functional domains in plant shoot meristems. U. Brand et al. 2001. Bioassays 23: 134-141.
- -Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by CLV3 activity.
 - U. Brand et al. 2000. Science 289: 617-619

5. Root development

Fasciation is normally a result from an increased size of the apical meristem in apical plant organs. Modulation of the number of cells within the proliferating zone of the root apical meristem results in an excess number of cellular divisions, giving rise to excess numbers of primordia formed or to roots in which the number of cells is increased. Adaptation to soil conditions is possible by regulation of 10 root development of plants. Here we describe several processes in root development that can be manipulated by modification of the levels of the RKS signaling complex within the root. The invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene 15 or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating root development, in particular wherein said gene comprises an ELS1, ELS2, RKS1, 20 RKS3, RKS4, RKS6 RKS8 or RKS10 gene or functional equivalent thereof. Root length, a result by either root cells proliferation or elongation, can for example be increased by overexpression of for example RKS3, RKS4, RKS6 and ELS2, or inactivation of the endogenous RKS10 gene product. Root length 25 can also be decreased by decreasing of endogenous RKS1 levels or by strong overexpression of RKS10. The initiation of lateral roots is also regulated by RKS gene products. Overexpression of for example RKS10 can result in a strong increase in the initiation and outgrowth of lateral roots. Co-30 suppression of RKS1 also resulted in the initiation and outgrowth of large numbers of lateral roots. Root hair formation and elongation is important in determining the total contact surface between plant and soil. A strong increase of root hair length (elongation) can be obtained by 35 overexpression of ELS1 and RKS3 gene products. As the roots of terrestrial plants are involved in the acquisition of water and nutrients, anchorage of the plant, synthesis of plant

hormones, interaction with the rhizosphere and storage functions, increasing or decreasing root length, for example for flexible adaptations to different water levels, can be manipulated by overexpressing or cosuppressing RKS and / or 5 ELS gene products. Modulation of the total contact surface between plant cells and the outside environment can be manipulated by regulation lateral root formation (increased by RKS10 overexpression and co-suppression of RKS1). Finally the contact surface between plant cells and the soil can be influenced by modulation of the number of root hairs formed or the elongation of the root hairs, as mediated by ELS1 and RKS3.

Results obtained

10

- 15 Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in Arabidopsis thaliana. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail.
- 20 T2 transgenic seedlings of Arabidopsis were germinated on MS agar plates without hormones. Control transgenic seedstocks containing a negative control vector pGreen4K (empty expression vector) and / or pGreen5K (a GUS overproducing vector) were included as references for normal root
- 25 development. Seedlings from transgenic Arabidopsis thaliana containing either constructs overexpressing (s) or cosuppressing by antisense (a) the RKS gene products were screened for the appearance of fasciation. Several examples in which fasciation could be routinely observed are shown
- together with a negative control plant (pGreen4K, containing 30 an expressing cassette without an insert cDNA). Seedlings are germinated and grown on vertically placed MS agar plates.

-Cellular organisation of the Arabidopsis thaliana root. L. Dolan et al. 1993. Development 119: 71-84

- -Root development in *Arabidopsis*: four mutants with dramatically altered root morphogenesis. P.N. Benfey et al.
- 5 1993. Development 119: 57-70

 -The development of plant roots: new approaches to underground problems. J.W. Schiefelbeim and P.N. Benfey 1991. the Plant

Cell 3: 1147-1154

6. Apical meristems

All parts of the plant above the ground are generally the result on one apical shoot meristem that has been initiated early at embryogenesis and that gives rise to all apical organs. This development of a single meristem into complex tissue and repeated patterns is the result of tissue and stage-dependent differentiation processes within the meristems and its resulting offspring cells. The control of meristem 10 formation, meristem identity and meristem differentiation is therefore an important tool in regulating plant architecture and development. Here we present evidence the function of RKS and ELS gene products in regulation of the meristem identity and the formation and outgrowth of new apical meristems. The 15 invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and 20 RKS/ELS ligand protein allowing modulating meristem identity, in particular wherein said gene comprises an ELS1, RKS8, RKS10 or RKS13 gene or functional equivalent thereof. Introduction of for example the RKS10 gene product or an other member of the RKS signaling complex under the control of a tissue and / 25 or stage specific promoter as provided herein allows localized and time regulated increases in the levels of gene product. For example the meristematic identity in a determined meristem might thereby be switched back into an undetermined meristem, thereby changing for example a terminal flower into an 30 undetermined generative meristem. Another application might be found in changing the meristematic identity at an early time point, during early vegetative growth, thereby switching the vegetative meristem into a generative meristem, allowing early flowering. 35 Modulation of meristem identity in terminal primordia, like for example as shown in Figure 30, where flower organ primordia are converted into terminal flower primordia, allows

the formation of completely new types of flowers and fused fruit structures. Constitutive overexpression of RKS gene products results in plants with many apical meristems, as can clearly been seen in Figure 29, where RKS10 overexpression results in an extremely bushy phenotype.

Results obtained

15

20

25

30

Changing the normal levels of endogenous RKS10 within the plant, either by overexpressing or co-suppressing the RKS10 cDNA, results in an increase in generative meristem development (Figure 28).

Compared with the control empty vector transgenic pGreen4K plants, large number of meristems are initiated at places were normally no meristems initiate and / or develop. A clear example is shown by co-suppressing the RKS8 gene (Figure 29), where many new inflorescence meristems are initiated from the central generative meristem compared with control pGreen4K plants of the same age. This phenotype is even more extreme in RKS10 overexpressing plants where the resulting plants are extremely bushy with very large numbers of generative meristems formed. Inactivation of the endogenous RKS10 gene in Arabidopsis results in modification of meristematic identity as can be shown in Figure 30. A determined flower meristem develops into two new normal terminal flower meristems and a number of terminal flower organ primordia. Another example is shown in Figure 31 where meristem determination is switched from a terminal flower meristem, that normally result only in the normal numbers of terminal organ primordia, towards a number of organ primordia, a new undetermined generative meristem that develop into normal flowers or in a new terminal

number of organ primordia, a new undetermined generative meristem that develop into normal flowers or in a new terminal flower meristem with developmental abnormalities. Only half of the terminal flower primordia develop normally while an extra structure arises resembling a new flower stem with a petal/stamen like organ. The few pollen detectable on this

structure (Figure 32) were able to pollinate a MS1 (male sterile) Arabidopsis flower. Figure 33 shows the meristematic

developmental switch from a terminal flower meristem into a new undetermined generative meristem, that gives rise to a new formation of another undetermined meristem, and several normal and abnormal terminal flowers. The abnormal flowers again show the fusion of different structures, in this case from sepals, petals and stamen together (Figure 34). Surprisingly, directly on the generative stem another structure, resembling a single stamen was detectable. All these data indicate that a decrease in RKS1 expression levels results in switches in the 10 meristematic identity. Meristems can switch forward and backward between developmental stages, indicating that RKS10 is normally involved in regulating the meristematic identity and the developmental order of meristematic development. RKS13 seems to be involved in similar processes, as can be concluded from the switches in flower meristematic outgrowths observed in figure 35. Modification of the expression levels of RKS1 also results in modified meristem identity. Suppression of endogenous RKS1 levels results in a developmental switching of generative meristems towards vegetative meristems, together 20 with other phenotypes (results not shown).

Literature

- -To be, or not to be a flower-control of floral meristem
 25 identity. H. Ma 1998. Trends in Genetics 14: 26-32
 -A genetic framework for floral patterning. F. Parcy et al.
 1998 Nature 395: 561-566
 - -Evolution of flowers and inflorescences. E.S. Coen and J.M. Nugent 1994. Development supplement 107-116
- 30 -Control of shoot cell fate: beyond homeoboxes. M. Tsiantis 2001. the Plant Cell 13: 733-738
 - -Floral induction and determinations: where is flowering controlled? F.D. Hempel et al. 2000. Trends in plant science 5: 17-21
- 35 -The Arabidopsis compact inflorescence genes: phase-specific growth regulation and the determination of inflorescence

architecture. L. Goosey and R. Sharrock 2001. the Plant Journal 26: 549-559.

thereof.

7. Male sterility

Male sterility is a highly desired trait in many plant species. For example, manipulation of pollen development is crucial for F1 hybrid seed production, to reduce labour costs and for the production of low-environmental impact genetically engineered crops. In order to produce hybrid seed from inbred plant lines, the male organs are removed from each flower, and pollen from another parent is applied manually to produce the hybrid seed. This labour-intensive method is used with a 10 number of vegetables (e.g. hybrid tomatoes) and with many ornamental plants. Transgenic approaches, in which one or more introduced gene products interfere with normal pollen initiation and development is therefore highly desired. Especially when the number of revertants (growing normal 15 pollen) is extremely low. Male sterility in plants is a desired trait that has been shown already in many plant species as a result of the inactivation of expression of a number of genes essential for proper stamen development, mitotic divisions in the pollen 20 stem cells, or male gametogenesis. A method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling 25 complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating pollen development, in particular wherein said gene comprises an ELS2 or RKS10 gene or functional equivalent

Here we present data that show that overexpression of gene products, like transmembrane receptor kinases (RKS) and extracellular proteins (ELS) can also result in the formation of male sterility. The ability to induce male sterility by overexpressing specific genes as provided herein allows the opportunity to produce transgenic overexpressing plants in which the pollen development is inhibited. Stable single copy homozygous integration of such overexpressing traits into the

plant genome will render such plants completely sterile, making them excellent material for the production of F1 hybrid seed. Furthermore, the combined integration of a male sterility inducing overexpressing gene coupled directly with another desired transgene result in transgenic plants which are unable to produce transgenic seed, making these transgenic plants excellent material for outside growth without problems affecting transgenic pollen spreading throughout the environment, thereby eliminating possible crosses with wild plant species or other non-transgenic crops. The 10 combination of a desired transgene flanked on both sites by different male-sterility inducing overexpressing genes would decrease the frequency of pollen formation to an extremely low level. An example is an overexpressing construct of RKS10 at the 5'end of integrated DNA fragment, the desired transgene 15 expression cassette in the middle and at the 3'end of the integrated DNA the ELS2 overexpressing construct. This complete DNA fragment is integrated into the genome by conventional techniques, like particle bombardment, Agrobacterium transformation etc. Another possible application 20 concerns the modification of pollen in ornamental plant species like lily, where the release of pollen from cut flowers can be avoided by making transgenic plants in which pollen development is initiated by release from the stamen is 25 prevented (a desired trait that can be obtained by overexpressing for example ELS2, resulting in partial pollen development). Hereby the ornamental value of the stamen with pollen is not lost, but release of pollen is inhibited.

30 Results obtained

Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in Arabidopsis thaliana. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail.

T2 transgenic seedlings of Arabidopsis were germinated on MS agar plates without hormones. Control transgenic plants

containing a negative control vector pGreen4K (empty expression vector) were included as references for normal stamen and pollen development. RKS10 and ELS2 resulted in sterile plants when overexpressed in Arabidopsis. Antisense RKS10 plants resulted in a strong reduction in the number of pollen formed (Figure 36). In order to determine whether pollen development itself was the reason for sterility (and not a combination of pollen developmental mutants coupled to either embryo lethals or female gametogenesis defects), reciprocal crosses were performed between sterile transgenic 10 plants and wildtype Arabidopsis thaliana WS plants. These results confirmed that the sterile plants with overexpressing RKS10 and ELS2 constructs were male sterile but completely female fertile. No defects could be observed in embryo development from crosses between female transgenic 15 overexpressors and male wildtype pollen (results not shown). Since both antisense and overexpressing constructs of the RKS10 gene showed defects in proper pollen development we conclude that normal levels of endogenous RKS10 gene product are essential for proper pollen formation, outgrowth and 20 differentiation. In the ELS2 overexpressing plants the initiation of pollen grains was not inhibited. However the proper development of pollen grains in full grown viable pollen was clearly inhibited .

25

30

Literature

-The Arabidopsis male sterility1 (MS1) gene is a transcriptional regulator of male gametogenesis, with homology to the PHD-finger family of transcription factors. Wilson et al. 2001. the Plant Journal 28: 27-39
-Transposon tagging of a male sterility gene in Arabidopsis.
Aarts et al. 1993. Nature 363: 715-717

126

8. Resistance mechanisms

Two-hybrid interaction experiments have already shown in vitro interaction between RKS and NDRO-NHL and members of the SBP/SPL family. Here we show that in vivo the individual components of this signalling cascade are regulating identical processes, as based on functional genomics on transgenics plants, overexpressing or co-suppressing single components or combinations of components in this transmembrane signalling complex.

Here we show a large number of new members of the NDR/NHL gene family and we postulate a function as syntaxins in the pathogen resistance:

15 At2g27080;

10

20

35

50

MAERVYPADS PPQSGQFSGN FSSGEFPKKP APPPSTYVIQ VPKDQIYRIP PPENAHRFEQ
LSRKKTNRSN CRCCFCSFLA AVFILIVLAG ISFAVLYLIY RPEAPKYSIE GFSVSGINLN
STSPISPSFN VTVRSRNGNG KIGVYYEKES SVDVYYNDVD ISNGVMPVFY QPAKNVTVVK
LVLSGSKIQL TSGMRKEMRN EVSKKTVPFK LKIKAPVKIK FGSVKTWTMI VNVDCDVTVD
KLTAPSRIVS RKCSHDVDLW **

At5g21130

MTVEKPQEMT GDTNSDGFLT NKDVHRIKHP SLDTNDSSSS RYSVDSQKSR IGPPPGTYVI KLPKDQIYRV PPPENAHRYE YLSRRKTNKS

25 CCRRCLCYSL SALLIIIVLA AIAFGFFYLV
YQPHKPQFSV SGVSVTGINL TSSSPFSPVI RIKLRSQNVK GKLGLIYEKG NEADVFFNGT
KLGNGEFTAF KQPAGNVTVI VTVLKGSSVK LKSSSRKELT ESQKKGKVPF GLRIKAPVKF

KVGSVTTWTM TITVDCKITV DKLTASATVK TENCETGLSL L*

30 At1g65690

MSQHQKIYPV QDPEAATARP TAPLVPRGSS RSEHGDPSKV PLNQRPQRFV PLAPPKKRRS
CCCRCFCYTF CFLLLLVVAV GASIGILYLV FKPKLPDYSI DRLQLTRFAL NQDSSLTTAF
NVTITAKNPN EKIGIYYEDG SKITVWYMEH QLSNGSLPKF YQGHENTTVI YVEMTGQTQN
ASGLRTTLEE QQQRTGNIPL RIRVNQPVRV KFGKLKLFEV RFLVRCGVFV DSLATNNVIK
IQSSSCKFRL RL*

At5q36970

MSDHQKIHPV SDPEAPPHPT APLVPRGSSR SEHGDPTKTQ QAAPLDPPRE KKGSRS CWCRCVCYTLLVLF LLIVIVGAIV GILYLVFRPK FPDYNIDRLQ LTRFQLNQDL

40 SLSTAFNVTI
TAKNPNEKIG IYYEDGSKIS VLYMQTRISN GSLPKFYQGH ENTTIILVEM TGFTQNATSL
MTTLQEQQRL TGSIPLRIRV TQPVRIKLGK LKLMKVRFLV RCGVSVDSLA ANSVIRVRSS
NCKYRFRL*

45 At1g54540

MGDQQKIHPV LQMEANKTKT TTPAPGKTVL LPVQRPIPPP VIPSKNRNMC CKIFCWVLSL LVIALIALAI AVAVVYFVFH PKLPSYEVNS LRVTNLGINL DLSLSAEFKV EITARNPNEK IGIYYEKGGH IGVWYDKTKL CEGPIPRFYQ GHRNVTKLNV ALTGRAQYGN TVLAALQQQQ QTGRVPLDLK VNAPVAIKLG NLKMKKIRIL GSCKLVVDSL STNNNINIKA SDCSFKAKL*

At5g06320

127

MADLNGAYYG PSIPPPKKVS HSHGRRGGGC GCLGDCLGCC GCCILSVIFN ILITIAVLLG
IAALIIWLIF RPNAIKFHVT DAKLTEFTLD PTNNLRYNLD LNFTIRNPNR RIGVYYDEIE
VRGYYGDQRF GMSNNISKFY QGHKNTTVVG TKLVGQQLVL LDGGERKDLN EDVNSQIYRI
DAKLRLKIRF KFGLIKSWRF KPKIKCDLKV PLTSNSTSGF VFOPTKCDVD F**

5

10

At5g11890

MTDRVFPASK PPTATNGAPP VGSIPPPPAP ATVTSNGTTN GMANQKPQVY IPANRPVYRP QPYSRRHHHQ SRPSCRRICC CCCFWSILII LILALMTAIA ATAMYVIYHP RPPSFSVPSI RISRVNLTTS SDSSVSHLSS FFNFTLISEN PNQHLSFSYD PFTVTVNSAK SGTMLGNGTV PAFFSDNGNK TSFHGVIATS TAARELDPDE AKHLRSDLTR ARVGYEIEMR TKVKMIMGKL KSEGVEIKVT CEGFEGTIPK GKTPIVATSK KTKCKSDLSV KVWKWSF*

At1g17620

MTDDRVYPAS KPPAIVGGGA PTTNPTFPAN KAQLYNANRP AYRPPAGRRR TSHTRG

15 CCCRCCCWTIFVII LLLLIVAAAS AVVYLIYRPQ RPSFTVSELK ISTLNFTSAV
RLTTAISLSV

IARNPNKNVG FIYDVTDITL YKASTGGDDD VVIGKGTIAA FSHGKKNTTT LRSTIGSPPD ELDEISAGKL KGDLKAKKAV AIKIVLNSKV KVKMGALKTP KSGIRVTCBG IKVVAPTGKK ATTATTSAAK CKVDPRFKIW KITF**

20

At3g11650

MGSKQPYLNG AYYGPSIPPP PKAHRSYNSP GFGCCCFSCL GSCLRCCGCC ILSLICNILI
AVAVILGVAA LILWLIFRPN AVKFYVADAN LNRFSFDPNN NLHYSLDLNF TIRNPNQRVG
VYYDEFSVSG YYGDQRFGSA NVSSFYQGHK NTTVILTKIE GQNLVVLGDG ARTDLKDDEK
SGIYRINAKL RLSVRFKFWF IKSWKLKPKI KCDDLKIPLG SSNSTGGFKF QPVQCDFDLS**

At2g22180

MEGPRRPPSA TAPDSDDDKP DDPPSVWHRP TSSLPALPSL DPPSHGSHHW RNHSLNLSPL
30 PTTSSPPLPP PDSIPELETY VVQVPRDQVY WTPPPEHAKY VEKRSKNPEK NKKKGCSKRL
LWFFIILVIF GFLLGAIILI LHFAFNPTLP VFAVERLTVN PSNFEVTLRA ENPTSNMGVR
YMMEKNGVVS LTYKNKSLGS GKFPGLSQAA SGSDKVNVKL NGSTKNAVVQ PRGSKQPVVL
MLNMELKAEY EAGPVKRNKE VVVTCDVKVK GLLDAKKVEI VSENCESEFK N*

35 At5g22870

MCHKPKLBLM PMETSPAQPL RRPSLICYIF LVILTLIFMA AVGFLITWLE TKPKKLRYTV ENASVQNFNL TNDNHMSATF QFTIQSHNPN HRISVYYSSV EIFVKFKDQT LAFDTVEPFH QPRMNVKQID ETLIAENVAV SKSNGKDLRS QNSLGKIGFE VFVKARVRFK VGIWKSSHRT AKIKCSHVTV SLSQPNKSQN SSCDADI*

40

45

At2g35980

MAAEQPLNGA FYGPSVPPPA PKGYYRRGHG RGCGCCLLSL FVKVIISLIV ILGVAALIFW LIVRPRAIKF HVTDASLTRF DHTSPDNILR YNLALTVPVR NPNKRIGLYY DRIEAHAYYE GKRFSTITLT PFYQGHKNTT VLTPTFQGQN LVIFNAGQSR TLNAERISGV YNIEIKFRLR VRFKLGDLKF RRIKPKVDCD DLRLPLSTSN GTTTTSTVFP IKCDFDF**

At2g46300

MADYQMNPVL QKPPGYRDPN MSSPPPPPPP IQQQPMRKAV PMPTSYRPKK KRRSCCRFCC CCICITLVLF IFLLLVGTAV FYLWFDPKLP TFSLASFRLD GFKLADDPDG ASLSATAVAR VEMKNPNSKL VFYYGNTAVD LSVGSGNDET GMGETTMNGF RQGPKNSTSV KVETTVKNQL VERGLAKRLA AKFQSKDLVI NVVAKTKVGL GVGGIKIGML AVNLRCGGVS LNKLDTDSPK CILNTLKWYK IISN*

At4g05220

55 MTPDRTTIPI RTSPVPRAQP MKRHHSASYY AHRVRESLST RISKFICAMF LLVLFFVGVI AFILWLSLRP HRPRFHIQDF

128

VVQGLDQPTG VENARIAFNV TILNPNQHMG VYFDSMEGSI YYKDQRVGLI PLLNPFFQQP TNTTIVTGTL TGASLTVNSN RWTEFSNDRA QGTVGFRLDI VSTIRFKLHR WISKHHRMHA NCNIVVGRDG LILPKFNHKR CPVYFT*

5 At2q35460

MANGLNGASY GPPIKPPVKT YYSHGRRGSD VGCGICGCFS SCLLCCGGCL VNIICNILIG VLVCLGVVAL ILWFILRPNV VKFQVTEADL TRFEFDPRSH NLHYNISLNF SIRNPNQRLG IHYDQLEVRG YYGDQRFSAA NMTSFYQGHK NTTVVGTELN GQKLVLLGAG GRRDFREDRR SGVYRIDVKL RFKLRPKFGF LNSWAVRPKI KCHLKVPLST SSSDERFQFH PTKCHVDL*

10

15

At2g27260

MQDPSRPATG YPYPYPYPNP QQQQPPTNGY PNPAAGTAYP YQNHNPYYAP QPNPRAVIIR RLFIVFTTFL LLLGLILFIF FLIVRPQLPD VNLNSLSVSN FNVSNNQVSG KWDLQLQFRN PNSKMSLHYE TALCAMYYNR VSLSETRLQP FDQGKKDQTV VNATLSVSGT YVDGRLVDSI GKERSVKGNV EFDLRMISYV TFRYGAFRRR RYVTVYCDDV AVGVPVSSGE GKMVGSSKRC KTY**

At4g01410

MGEGEAKAEH AAKADHKNAP SASSTPESYS KEGGGGGGDA RRAICGAIFT ILVILGIIAL

20 ILWLVYRPHK PRLTVVGAAI YDLNFTAPPL ISTSVQFSVL ARNPNRRVSI HYDKLSMYVT
YKDQIITPPL PLPPLRLGHK STVVIAPVMG GNGIPVSPEV ANGLKNDBAY GVVLMRVVIF
GRLRWKAGAI KTGRYGFYAR CDVWLRFNPS SNGQVPLLAP STCKVDV*

At5g22200

25 MTGRYCDQHN GYEERRMRMM MRRIAWACLG LIVAVAFVVF LVWAILHPHG PRFVLQDVTI
NDFNVSQPNF LSSNLQVTVS SRNPNDKIGI FYDRLDIYVT YRNQEVTLAR LLPSTYQGHL
EVTVWSPFLI GSAVPVAPYL SSALNEDLFA GLVLLNIKID GWVRWKVGSW VSGSYRLHVN
CPAFITVTGK LTGTGPAIKY QLVQRCAVDV *

30 At1g61760

MHNKVDSLPV RSNPSTRPIS RHHSASNIVH RVKESLTTRV SKLICAIFLS LLLCLGIITF ILWISLQPHR PRVHIRGFSI SGLSRPDGFE TSHISFKITA HNPNQNVGIY YDSMEGSVYY KEKRIGSTKL TNPFYQDPKN TSSIDGALSR PAMAVNKDRW MEMERDRNQG KIMFRLKVRS MIRFKVYTWH SKSHKMYASC YIEIGWDGML LSATKDKRCP VYFT*

35

At3g52470

MSKDCGNHGG GKEVVVRKLC AAIIAFIVIV LITIFLVWVI LRPTKPRFVL QDATVYAFNL SQPNLLTSNF QVTIASRNPN SKIGIYYDRL HVYATYMNQQ ITLRTAIPPT YQGHKEVNVW SPFVYGTAVP IAPYNSVALG EEKDRGFVGL MIRADGTVRW KVRTLITGKY HIHVRCQAFI

40 NLGNKAAGVL VGDNAVKYTL ANKCSVNV**

At5g53730

MSQISITSPK HCAKKGGINI NNRHKKLFFT FSTFFSGLLL IIFLVWLILH PERPEFSLTE
ADIYSLNLTT SSTHLLNSSV QLTLFSKNPN KKVGIYYDKL LVYAAYRGQQ ITSEASLPPF

45 YQSHEEINLL TAFLQGTELP VAQSFGYQIS RERSTGKIII GMKMDGKLRW KIGTWVSGAY
RFNVNCLAIV AFGMNMTIPP LASLQGTRCS TTI*

At4g01110

MAGETLLKPV LQKPPGYREL HSQPQTPLGS SSSSSSMLRR PPKHAIPAAF YPTKKRQWSR
CRVFCCCVCI TVAIVILLLI LTVSVFFLYY SPRLPVVRLS SFRVSNFNFS GGKAGDGLSQ
LTAEATARLD FRNPNGKLRY YYGNVDVAVS VGEDDFETSL GSTKVKGFVE KPGNRTVVIV
PIKVKKQQVD DPTVKRLRAD MKSKKLVVKV MAKTKVGLGV GRRKIVTVGV TISCGGVRLQ
TLDSKMSKCT IKMLKWYVPI QVKCI*

55 At2g35960

MTTKDCGNHG GGGGGGTASR ICGVIIGFII IVLITIFLVW IILQPTKPRF ILQDATVYAF

129

NLSQPNLLTS NFQITIASRN RNSRIGIYYD RLHVYATYRN QQITLRTAIP PTYQGHKEDN VWSPFVYGNS VPIAPFNAVA LGDEQNRGFV TLIIRADGRV RWKVGTLITG KYHLHVRCQA FINLADKAAG VHVGENAVKY MLINKCSVNV *

5 At3g52460

MPSPPEETQ PKPDTGPGQN SERDINQPPP PPPQSQPPPP QTQQQTYPPV MGYPGYHQPP
PPYPNYPNAP YQQYPYAQAP PASYYGSSYP AQQNPVYQRP ASSGFVRGIF TGLIVLVVLL
CISTTITWLV LRPQIPLFSV NNFSVSNFNV TGPVFSAQWT ANLTIENQNT KLKGYFDRIQ
GLVYHQNAVG EDBFLATAFF QPVFVETKKS VVIGRTLTAG DKEQPKVPSW VVDEMKKERE
10 TGTVTFSLRM AVWVTFKTDG WAARESGLKV FCGKLKVGFE GISGNGAVLL PKPLPCVVYV*

MTTKECGNHG GGGGGGTAC RICGAIIGFI IIVLMTIFLV WIILQPKNPE FILQDTTVYA
FNLSQPNLLT SKFQITIASR NRNSNIGIYY DHLHAYASYR NQQITLASDL PPTYQRHKED
SVWSPLLYGN QVPIAPFNAV ALGDEQNSGV FTLTICVDGQ VRWKVGTLTI GNYHLHVRCQ
AFINQADKAA GVHVGENTVK YTLINKCSVN F*

At2g35970

At4g09590

MTTKECGNHG GGGGGGTAC RICGAIIGFI IIVLMTIFLV SIILQPKKPE FILQDTTVYA

20 FNLSQPNLLT SKFQITIASR NRNSNIGIYY DHLHAYASYR NQQITLASDL PPTYQRHKEN
SVWSPLLYGN QVPIAPFNAV ALGDEQNSGV FTLTICVDGR VRWKVGTLTI GNYHLHVRCQ
AFINQADKAA GVHVGENTVK YTLINKCSVN F*

At3g26350

MSHHHHHETN PHFARIPSON PHLKSGGAST SQTSSNQPHI PPIPHPKKSH HKTTQPHPVA
PPGILIKTRG RHRENPIQEP KHSVIPVPLS PEERLPPRKT QNSSKRPLLL SPEDNQQQRP
PPPQAPQRNG GGYGSTLPPI PKPSPWRTAP TPSPHHRRGP RLPPPSRETN AMTWSAAFCC
AIFWVILILG GLIILIVYLV YRPRSPYVDI SAANLNAAYL DMGFLLNGDL TILANVTNPS
KKSSVEFSYV TFELYYYNTL IATQYIEPFK VPKKTSMFAN VHLVSSQVQL QATQSRELQR
30 QIETGPVLLN LRGMFHARSH IGPLFRYSYK LHTHCSVSLN GPPLGAMRAR RCNTKR*

At3g11660

MKDCENHGHS RRKLIRRIFW SIIFVLFIIF LTILLIWAIL QPSKPRFILQ DATVYAFNVS
GNPPNLLTSN FQITLSSRNP NNKIGIYYDR LDVYATYRSQ QITFPTSIPP TYQGHKDVDI
35 WSPFVYGTSV PIAPFNGVSL DTDKDNGVVL LIIRADGRVR WKVGTFITGK YHLHVKCPAY
INFGNKANGV IVGDNAVKYT FTTSCSVSV**

At3g44220

MTEKECEHHH DEDEKMRKRI GALVLGFLAA VLFVVFLVWA ILHPHGPRFV

40 LQDATIYAFN VSQPNYLTSN LQVTLSSRNP NDKIGIFYDR LDIYASYRNQ
QVTLATLLPA TYQGHLDVTI WSPFLYGTTV PVAPYFSPAL SQDLTAGMVL
LNIKIDGWVR WKVGTWVSGR YRLHVNCPAY ITLAGHFSGD GPAVKYOLVO RCAVDV*

At1g08160

MVPPNPAHQP ARRTQPQLQP QSQPRAQPLP GRRMNPVLCI IVALVLLGLL VGLAILITYL TLRPKRLIYT VEAASVQEFA IGNNDDHINA KFSYVIKSYN PEKHVSVRYH SMRISTAHHN QSVAHKNISP FKQRPKNETR IETQLVSHNV ALSKFNARDL RAEKSKGTIE MEVYITARVS YKTWIFRSRR RTLKAVCTPV MINVTSSSLD GFQRVLCKTR L**

50 At2g01080

MPPPPSSRA GLNGDPIAAQ NQQPYYRSYS SSSSASLKGC CCCLFLLFAF LALLVLAVVL IVILAVKPKK PQFDLQQVAV VYMGISNPSA VLDPTTASLS LTIRMLFTAV NPNKVGIRYG ESSFTVMYKG MPLGRATVPG FYQDAHSTKN VEATISVDRV NLMQAHAADL VRDASLNDRV ELTVRGDVGA KIRVMNFDSP GVQVLLPSFL PAFCSLSDLA *

55

At5g06330

130

MTSKDCGSHD SHSSCNRKIV IWTISIILLL ILVVILLVWA ILQPSKPRFV LQDATVFNFN VSGNPPNLLT SNFQFTLSSR NPNDKIGIYY DRLDVYASYR SQQITLPSPM LTTYQGHKEV NVWSPFVGGY SVPVAPYNAF YLDQDHSSGA IMLMLHLDGR VRWKVGSFIT GKYHLHVRCH ALINFGSSAA GVIVGKYMLT ETCSVSV*

5

At5g56050

MSKFSPPPQS QPQPPETPPW ETPSSKWYSP IYTPWRTTPR STQSTPTTTP IALTEVIVSK SPLSNQKSPA TPKLDSMEAH PLHETMVLLQ LRTSRTNPWI WCGAALCFIF SILLIVFGIA TLILYLAVKP RTPVFDISNA KLNTILFESP VYFNGDMLLQ LNFTNPNKKL NVRFENLMVE LWFADTKIAT QGVLPFSQRN GKTRLEPIRL ISNLVFLPVN HILELRRQVT SNRIAYEIRS NFRVKAIFGM IHYSYMLHGI CQLQLSSPPA GGLVYRNCTT KRW*

At3g20600

NDR1

MNNQNEDTEG GRNCCTCCLS FIFTAGLTSL FLWLSLRADK PKCSIQNFFI PALGKDPNSR
DNTTLNFMVR CDNPNKDKGI YYDDVHLNFS TINTTKINSS ALVLVGNYTV PKFYQGHKKK
AKKWGQVKPL NNQTVLRAVL PNGSAVFRLD LKTQVRFKIV FWKTKRYGVE VGADVEVNGD
GVKAQKKGIK MKKSDSSFPL RSSFPISVLM NLLVFFAIR*

20 At3q54200

MSDFSIKPDD KKEEEKPATA MLPPPKPNAS SMETQSANTG TAKKLRKRN CKICICFTIL LILLIAIVIV ILAFTLFKPK RPTTTIDSVT VDRLQASVNP LLLKVLLNLT LNVDLSLKNP NRIGFSYDSS SALLNYRGQV IGEAPLPANR IAARKTVPLN ITLTLMADRL LSETQLLSDV MAGVIPLNTF VKVTGKVTVL KIFKIKVQSS SSCDLSISVS DRNVTSQHCK YSTKL*

25

30

At3g20590

non-race specific disease resistance protein, putative
MTKIDPEEL GRKCCTCFFK FIFTTRLGAL ILWLSLRAKK PKCSIQNFYI PALSKNLSSR
DNTTLNFMVR CDNPNKDKGI YYDDVHLTFS TINTTTTNSS DLVLVANYTV PKFYQGHKKK
AKKWGQVWPL NNQTVLRAVL PNGSAVFRLD LKTHVRFKIV FWKTKWYRRI KVGADVEVNG
DGVKAQKKGS KTKKSDSSLP LRSSFPIFVL MNLLVFFAIR *

At4g39740

MSHVTATSLA RFTKPVPKPA SSPIVNTKLT TSGGRTAAFM DLSSFRLTVW

DPDTANDSSG KFPWPRFLFF FLTLKTGGSG LNIKPTISAI AQMMNPMTIT

EMNNQMHRLE QKLLLFLPGS LFLRLSTILH YPGEGSNRPD PLEHALRRSR

SLGLDQEEAA KKVIRVGRDS KNDYVNVVEN QAASFLRRCG PSKRIQSVNY

CKSTRQGHEI PDVKPLFPTG GGTQAPSRSR ARYAVPAILL GFAGFVGFLH

YNDERRAVPR GQASSNSGCG CGSNTTVKGP IIGGPFTLVS TENKIVTEND

FCGKWVLLYF GYSFSPDVGP EQLKMMSKAV DKLAILLNPL TFGCLYLYAE

FDSRILGLTG TASAMRQMAQ EYRVYFKKVQ EDGEDYLVDT SHNMYLINPK

MEIVRCFGVE YNPDELSQEL LKEVASVSO*

At1g32270 syntaxin, putative

45 MVRSNDVKFQ VYDAELTHFD LESNNNLQYS LSLNLSIRNS KSSIGIHYDR
FEATVYYMNQ RLGAVPMPLF YLGSKNTMLL RALFEGQTLV LLKGNERKKF
EDDQKTGVYR IDVKLSINFR VMVLHLVTWP MKPVVRCHLK IPLALGSSNS
TGGHKKMLLI GQLVKDTSAN LREASETDHR RDVAQSKKIA DAKLAKDFEA
ALKEFQKAQH ITVERETSYI PFDPKGSFSS SEVDIGYDRS QEQRVLMESR
RQEIVLLDNE ISLNEARIEA REQGIQEVKH QISEVMEMFK DLAVMVDHQG
TIDDIDEKID NLRSAAAQGK SHLVKASNTQ GSNSSLLFSC SLLLFFFLSG
DLCRCVCVGS ENPRLNPTRR KAWCEEEDEE QRKKQQKKKT MSEKRREEK
KVNKPNGFVF CVLGHK*

MSHHHYETNP HFVQFSLQDQ HQGGPSSSWN SPHHHQIPQA HSVAPPRVKI KTRGRHQTEP
PETIHESPSS RPLPLRPEEP LPPRHNPNSA RPLQLSPEEQ RPPHRGYGSE PTPWRRAPTR
PAYQQGPKRT KPMTLPATIC CAILLIVLIL SGLILLLVYL ANRPRSPYFD ISAATLNTAN
LDMGYVLNGD LAVVVNFTNP SKKSSVDFSY VMFELYFYNT LIATEHIEPF IVPKGMSMFT
SFHLVSSQVQ IQMIQSQDLQ LQLGTGPVLL NLRGTFHARS NLGSLMRYSY WLHTQCSISL
NTPPAGTMRA RRCNTKR*

At5q45320

MPRLTSRHGT SPFIWCAAII CAIISIVVIV GGIIVFVGYL VIHPRVPIIS

VADAHLDFLK YDIVGVLQTQ LTIVIRVEND NAKAHALFDE TEFKLSYEGK
PIAILKAPEF EVVKEKSMFL PYLVQSYPIP LNPTMMQAVD YAVKKDVITF
ELKGGSRTRW RVGPLGSVKF ECNLSCQLRF RPSDHSYIPS PCTSAHKH*

At3g20610

MDRDDAWEWF VTIVGSLMTL LYVSFLLALC LWLSTLVHHI PRCSIHYFYI PALNKSLISS DNTTLNFMVR LKNINAKQGI YYEDLHLSFS TRINNSSLLV ANYTVPRFYQ GHEKKAKKWG QALPFNNQTV IQAVLPNGSA IFRVDLKMQV KYKVMSWKTK RYKLKASVNL EVNEDGATKV KDKEDGIKMK ISDSSPQRLT FFQVCFSIIC VLMNWLIFLA IR*

20 At4g26490

MVLTKPATVR FNGLDAEPRK DRVILRQPRS SRTSLWIWCV AVFLAIRPRI PVFDIPNANL HTIYFDTPEF FNGDLSMLVN FTNPNKKIEV KFEKLRIELF FFNRLIAAQV VQPFLQKKHE TRLEPIRLIS SLVGLPVNHA VELRRQLENN KIEYEIRGTF KVKAHFGMIH YSYQLHGRCQ LOMTGPPTGI LISRNCTTKK *

25

30

At5g42860

MHAKTDSEVT SLSASSPTRS PRRPAYFVQS PSRDSHDGEK TATSFHSTPV
LTSPMGSPPH SHSSSSRFSK INGSKRKGHA GEKQFAMIEE EGLLDDGDRE
QEALPRRCYV LAFIVGFSLL FAFFSLILYA AAKPQKPKIS VKSITFEQLK
VQAGQDAGGI GTDMITMNAT LRMLYRNTGT FFGVHVTSSP IDLSFSQITI
GSGSIKKFYQ SRKSQRTVVV NVLGDKIPLY GSGSTLVPPP PPAPIPKPKK
KKGPIVIVEP PAPPAPVPMR LNFTVRSRAY VLGKLVQPKF YKRIVCLINF
EHKKLSKHIP ITNNCTVTSI *

35 At1g45688

MHAKTDSEVT SLAASSPARS PRRPVYYVQS PSRDSHDGEK TATSFHSTPV LSPMGSPPHS HSSMGRHSRE SSSSRFSGSL KPGSRKVNPN DGSKRKGHGG EKQWKECAVI EEEGLLDDGD RDGGVPRRCY VLAFIVGFFI LFGFFSLILY GAAKPMKPKI TVKSITFETL KIQAGQDAGG VGTDMITMNA TLRMLYRNTG TFFGVHVTST PIDLSFSQIK IGSGSVKKFY QGRKSERTVL VHVIGEKIPL YGSGSTLLPP APPAPLPKPK KKKGAPVPIP DPPAPPAPVP MTLSFVVRSR AYVLGKLVQP KFYKKIECDI NFEHKNLNKH IVITKNCTVT TV*

At4q26820

MDDEQNLVEE MNQQLLITVI DTEKVPELRP ISSRSHQESE PANISHWSLL FKLFLAITIM
GACVAGVTFV ILITPTPPTV HVQSMHISFA NHNLPVWSAT FSIKNPNEKL HVTYENPSVW
LVHRGKLVST ARADSFWQKG GEKNEVIVKR NETKVIDEEA AWEMEDEVAV TGGVVGLDMV
FSGRVGFYPG TSALWGEQYM SAVCENVSAK LYNVDDEIYG TNRSVLSFDG RLVCSVRLPK
YP*

50

Plants respond in a variety of ways to pathogens. After a recognition of the pathogen, normally mediated by avr and R genes, the resulting response induces a hypersensitive

response, that results in inhibition of the pathogen. After the recognition, further processes appear to be non-specific. In addition to the hypersensitive response, a second line of defence, defined as the systemic acquired resistance response can be triggered, that renders unaffected parts of the plant resistant to a variety of normally virulent pathogens. Several of the RKS and ELS gene products prove to be key regulators in the regulation of the system acquired resistance response.

- Overexpression of several of the RKS and / or ELS genes in plants, either by constitutive promoters, stage and / or tissue specific promoters, or inducible promoters allows the activation of a systemic acquired resistance response in plants.
- Another application can be provided by the activation of a RKS /ELS specific ligand in (transgenic) plants, thereby activating the receptor complex, that finally results in triggered activation of the systemic acquired resistance response in these plants.
- (ref. Generation of broad-spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. H. Cao et al. 1998. Proc. Natl. Acad. Sci. USA 95: 6531-6536). Recent literature shows the functional interaction between RKS10 and BRI-1, another class
 - of transmembrane LRR receptor kinases (Cell Vol. 110, 213-222 2002). BAK1=RKS10 as descibed here, interacts with BRI-1 and modulates brassinosteroid signaling; Cell vol 110, 203-212 2002 BRI1/BAK1 a receptor kinase pair mediating brassinosteroid signaling). Brassinosteroids are known to
- function in a broad range of disease resistance in tobacco and rice (Plant Journal 2003, 887-898). The BRI-1 receptor is involved in the binding of systemin, an 18 amino acid polypeptide, representing the primary signal for the systemic activation of defence genes (PNAS 2002, 9585-9590).
- 35 ELS overexpression phenotypes mimic the effects of inactivation of RKS molecules gene products. Either ELS is competing for ligand binding, or ELS inhibits the interactions

between RKS and BRI-1-like gene products. ELS1 overexpression results in dwarf phenotypes in Arabidopsis and tobacco plants, similar as observed for antisense RKS4 and RKS10, and for knock out plants of RKS0 and RKS4.

Deregulating expression of ELS and / or RKS genes in plant would modify the broad spectrum disease resistance in such plants. This would explain the observed data that brassinosteroids are involved in disease resistance (Plant Journal 2003, 33 887-898.)

10

Further references

Plant Journal 1997: 12, 2 367-377 Mol. Gen. Genet. 1996: 250, 7-16

5 Gene 1999, 237, 91-104

Genes and Development 1997: 11, 616-628

Proc. Natl. Acad. Sci. USA 1998: 95, 10306-10311

Plant Journal 2000: 22, 523-529

Science 1997: 278, 1963-1965

10 Plant Physiol. Biochem. 2000: 38, 789-796

Cell 1996: 84, 61-71

Annu. Rev. Plant Physiol. Plant Mol. Biol. 1999: 50, 505-537

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

= 010000 111 UIO 1111 BOO MOTAGO OUT UIO 1101 IIO 110 110 IIO 110 IIO 110 IIO IIO IIO IIO IIO IIO IIO IIO IIO II
☐ BLACK BORDERS
☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
☐ FADED TEXT OR DRAWING
☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
☐ SKEWED/SLANTED IMAGES
☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
☐ GRAY SCALE DOCUMENTS
☐ LINES OR MARKS ON ORIGINAL DOCUMENT
☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY

IMAGES ARE BEST AVAILABLE COPY.

☐ OTHER:

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.